A STUDY OF WINTER DDT HOUSE-SPRAYING AND ITS CONCOMITANT EFFECT ON ANOPHELINES AND MALARIA IN AN ENDEMIC AREA*

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(Received for Publication 5 February 1946)

In view of the potentiality of the lasting qualities of DDT, this study was planned to determine what effect the elimination of hibernating anopheline females would have on the mosquito population of a given area during the following season. This was to be accomplished solely by winter spraying of all adult resting places. Furthermore, the study was designed to answer the question as to whether one complete treatment in the absence of larval control would so reduce the mosquito population that malaria transmission would cease or at least be reduced to an insignificant level.

The Bonifica di Castelvolturno, about thirty miles north of Naples, Italy, was chosen as the experimental area. In addition to being close to Naples the Castelvolturno area was selected because the Malaria Demonstration Unit, Allied Commission, had carried on DDT house-spraying and dusting studies in the bonifica during the 1944 season and had amassed a great deal of important data regarding: (1) The degree of malaria endemicity (parasite and spleen surveys were made in May, August, and October); (2) the species of anophelines existing in the area; (3) the fluctuation in numbers of adult mosquitoes in treated and untreated shelters (based on weekly inspections); and (4) the lasting effects of single applications of 5 percent DDT in kerosene.

For purposes of comparison, observations were made in an untreated area in the Sessa Bonifica lying in the Garigliano River valley just to the north of the Volturno River.

Description of the Area

The Bonifica di Castelvolturno occupies the coastal portion of a wide plain lying at the mouth of the Volturno River (see accompanying map). Whereas the 1944 experimental area included only a portion of the bonifica south of the river approximately six miles long by three miles wide, it was felt that more conclusive results

* The writer wishes to express his deep appreciation to the following individuals without whose wholehearted cooperation this study could not have been attempted: Colonel W. S. Stone, M.C. (Chief, Preventive Medicine Section, Office of the Surgeon, MTOUSA), Dr. F. L. Soper, Dr. H. W. Kumm, Dr. L. A. Riehl and Mr. F. W. Knipe (all of the International Health Division of the Rockerfeller Foundation), Captain L. M. Klashman, Sn.C. and Sgt. C. R. Collins (137th Malaria Control Det.), Captain R. A. Fisher, Sn.C., 2nd Lt. A. W. Zeigler, Sn.C. and Tec. 4th C. S. Black, Jr. (all of the 15th Medical Laboratory).

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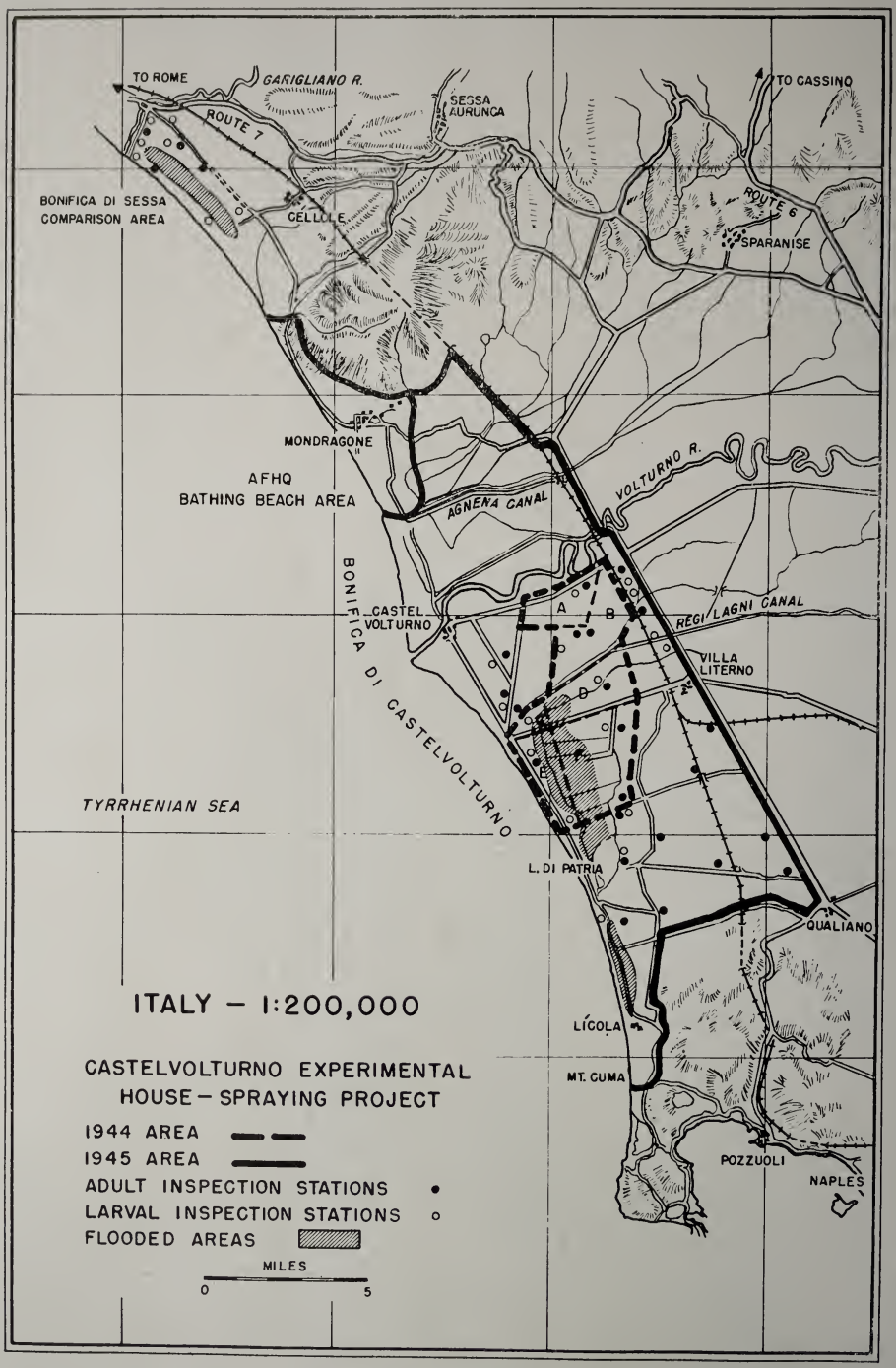
TO ROME
GARIGLIANO R.
ROUTE 7
SESSA AURUNCA
TO CASSINO
BONIFICA DI SESSA COMPARISON AREA
CELLOLE
ROUTE 6
SPARANISE
MONDRAGONE
AFHQ BATHING BEACH AREA
AGNENA CANAL
VOLTURNO R.
BONIFICA DI CASTELVOLTURNO
CASTEL VOLTURNO
REGI LAGNI CANAL
VILLA LITERNO
TYRRHENIAN SEA
L. DI PATRIA
QUALIANO
ITALY — 1:200,000
LICOLA
MT. CUMA
CASTELVOLTURNO EXPERIMENTAL HOUSE — SPRAYING PROJECT
1944 AREA
1945 AREA
ADULT INSPECTION STATIONS
LARVAL INSPECTION STATIONS
FLOODED AREAS
POZZUOLI
NAPLES
MILES
0
5

could be obtained if the test area was expanded. It was therefore decided that the enlarged area, which was bounded on the west by the Tyrrhenian Sea, should consist of a coastal strip approximately five miles wide, extending from the Mondragone hills south to the village of Qualiano, thence following the hills in a southwesterly fashion to Mt. Cuma. Included in this zone of approximately 93 square miles are two additional bonifiche, namely, the Bonifica di Varcaturo and the Bonifica di Lícola, also the towns of Mondragone ,population approximately 15,000), Castelvolturno population approximately 1,500) and Villa Literno (population approximately 5,000).

The area in general is thickly interspersed with canals and ditches, most of which had seen little maintenance in recent years and as a result were choked with vegetation; some ditch cleaning was accomplished in 1944 but nothing was done in 1945. A few artesian wells also existed in the area. Destruction of one pumping station in the Lícola-Varcaturo area and three stations in the Castelvolturno area by the retreating Germans late in September of 1943 caused two marshy lakes to be formed with the resultant flooding of many houses. Fig. 1. The lake in the Varcaturo Bonifica which was approximately a half mile wide and three miles long, remained in continuous existence for almost two years; on the 18th of July 1945 the reconstructed pumping station commenced operations and within three weeks the marsh was practically dry. The inundated area in the Castelvolturno Bonifica, which was approximately one and a half miles wide and four and a half miles long, became dry both summers as the result of rehabilitating one of the pumping stations. Unfortunately this station did not have the capacity to compete with heavy winter rains and as a result the reclaimed farmlands were flooded each winter. During the 1945 season, the lake had disappeared by the first week in June.

Perhaps the driest part of the experimental area is the southeastern corner from the shore of Lago di Patria east of Qualiano where the land rises slightly, affording good drainage. Throughout this section one finds the old style of Italian farm house, consisting of several floors and many rooms and stables with very high ceilings, all of which tended to make house-spraying very difficult. On the other hand the bonifica houses are modern and simply constructed; treating these was an easy matter.

During the winter months of 1945 experimental activities were handicapped by cold, rainy weather, bad roads (some impassable) and an abundance of mud. With the coming of summer the

mud turned to deep drifts of dust. A hot, dry season eliminated many aquatic situations; however, the principal drainage canals and many of the smaller ditches contained water and were heavily overgrown with algae, duck weed and other vegetation, providing ideal anopheline larval habitats. The following table gives local weather data, which of necessity had to be taken from Capodichino Airfield, Naples. It will be noticed that temperatures tended to be somewhat higher and rainfall slightly less in 1945.

Table 1.—Temperatures in Degrees Fahrenheit and Rainfall in Inches for the Naples area for 1944 and 1945.

Month	Ave. Max. Temp.		Ave. Min. Temp.		Mean Temp.		Rainfall	
	1944	1945	1944	1945	1944	1945	1944	1945
January	53	49	39	36	46	42	4:20	11.37
February	51	56	39	40	45	48	4.99	0.91
March	54	61	41	43	48	52	3.79	0.20
April	69	68	52	50	60	59	2.05	0.54
May	75	78	57	56	66	67	1.18	2.84
June	80	85	60	65	70	75	1.41	Trace
July	85	89	65	67	75	78	0.15	0.20
August	89	87	69	67	79	----	0.08	0.59
September	81	79	61	61	71	----	7.08	5.26
October	70	----	53	----	62	----	12.35	------
November	61	----	47	----	54	----	5.62	------
December	55	----	43	----	49	----	3.02	------

Figure 1. *View of typical Bonifica house in the flooded varcaturo-Licola area.* (Photo by J. M. Andrews.)

The 1944 *Season*

The following is a brief review of the activities and accomplishments of the Malaria Demonstration Unit, Allied Commission, in the Castelvolturno area during 1944. A complete report has been prepared by that organization and submitted elsewhere.

Application of insecticide and its effectiveness — Original applications in May were directed towards determining the relative merits of spraying with 5 percent DDT in kerosene and dusting with 10 percent DDT in powder (pyrophylite). Zones B and D received an application of oil, E and F were treated with dust and A and C remained as untreated controls (see map). Beacuse of the rather poor results obtained with powder, houses which had received this treatment were again dusted in July. In August the entire bonifica of 214 houses (all six zones) was sprayed with 5 percent DDT in kerosene, except for two test bedrooms in Zone B, which received only the original spraying.

DDT spray was applied with a hand-operated knapsack sprayer having a Bordeau nozzle. Walls and ceilings were treated at the rate of 60 milligrams of DDT per square foot of sprayed surface, or one quart per 1,000 square feet. In August when the bonifica was resprayed 328 pounds of DDT were required to treat the 214 houses.

Effectiveness of the applications was determined both by the daily liberation of 100 mosquitoes in ventilated (screened windows) and unventilated (glass windows closed) bedrooms (previously sprayed) and counting the dead on the following day, and by making weekly catches in survey stations (sprayed and untreated controls).

The August report of the Malaria Demonstration Unit, Allied Commission, records 100 percent kills in unventilated rooms 105 days after spraying and around 90 percent kills in ventilated rooms during the same period. The results obtained in unventilated rooms are not particularly significant, as similar mortality of mosquitoes was noted in unventilated rooms which had not been sprayed, suggesting that factors of temperature and humidity were involved. It is known that effective control was still being obtained in ventilated rooms in October, approximately five months after spray application.

Effective control of adult mosquitoes naturally entering human habitations and stables (as contrasted with the "100 liberated mosquitoes technique" results mentioned above) was obtained for at least 80 days after treatment. During a visit to the bonifica on the 13th

Table 2.—Spleen Indices in Castelvolturno and Sessa Areas (1944-45)

Locality and Date	No. Exam.	Spleen Index	Percent of Spleens					Average Spleen	
			O	P	1	2	3 & 4	Value	Position
Castelvolturno Bonifica									
May 1944	485	43%	57	32	10	1	0	1.6	P
August	575	43	57	28	13	2	0	1.6	P
October	533	39	61	22	14	3	0	1.6	P
January 1945	355	27	74	15	10	1	0	1.4	P
May	410	42	58	30	11	1	0	1.6	P
August	423	25	75	20	5	0	0	1.2	P
Castelvolturno Town									
January 1945	94	22	78	13	6	3	0	1.3	P
May	104	39	61	26	10	3	0	1.6	P
August	159	25	75	21	4	0	0	1.3	P
Sessa Bonifica and Céllole Town									
May 1945	228	56	44	26	22	8	0	1.9	P
August	210	63	37	22	27	12	2	2.2	1

of December, approximately four months after DDT application, no mosquitoes could be found in several of the treated houses examined, whereas 51 anophelines were counted in 15 minutes in a nearby untreated house, indicating that hibernating anophelines were present in the area but were restricted to unsprayed shelters.

Entomological findings (anopheline species) — Anopheline species encountered in the area consisted of: *Anopheles labranchiae* Falleroni, *A sacharovi* Farr, *A. melanoon* Hackett, and *A. messeae* Falleroni. Whereas *A. labranchiae* was the dominant species early in the year, surveys in August indicated that *A. sacharovi* accounted for 80 percent of the captured anophelines.

Degree of malaria endemicity — Of considerable interest to the 1945 project were the three spleen and parasite surveys conducted during May, August and October of the previous season. Only children residing in the bonifica between the ages of 5 and 15 years were examined. Fortunately, it was possible to utilize the services of the same doctors in those as well as subsequent surveys, thus enhancing the value of the spleen palpations.

The May survey which was initiated prior to the house spraying presents an index of the malaria endemicity of the area before control measures were adopted (see Tables 2 and 3). It is of interest to note the rapid drop in the parasite index from 21 percent to 8 percent during the three and a half month period following the application of DDT.

Table 3—Parasite Indices in Castelvolturno and Sessa Areas (1944-45)

Locality and Date	Number Examined	Number with Parasites	Percent with Parasites
Castelvolturno Bonifica			
May — 1945	485	101	21%
August	575	67	12
October	533	41	8
January — 1945	355	10	3
May	410	20	5
August	423	6	1
Castelvolturno Town			
January— 1945	94	2	2
May	104	7	7
August	159	12	8
Sessa Bonifica and Céllole Town			
May — 1945	228	40	18
August	210	87	41

The 1945 Season

House-spraying activities — Preparation of a 5 percent solution of DDT in kerosene was initiated by the 137th Malaria Control Detachment in Naples late in December 1944. Mixing was accomplished by rolling 50 gallon oil drums back and forth during the hottest part of the day. That complete solution was obtained is evidenced by the fact that at no time was a DDT sludge found at the bottom of the drums.

Spraying activities were commenced by the 137th M.C.D. on the 5th of January, and treatment of the 93-square-mile area was completed during the first week of April. An area of about eight square miles around the Mondragone bathing beach was treated by an Allied Force Headquarters team. Every house, stable, pigsty, cellar and grass shelter that it was possible to find was treated. In cases where houses were flooded, access was obtained by boat, but it became necessary to return at a later date, following the recession of the waters, to give the ground floors a proper spraying. In addition, there were instances where certain rooms were locked, and because no key was readily available, treatment had to be deferred to a later date. These temporarily unsprayed rooms provided sanctuary for mosquitoes, which resulted in abortive increases in mosquito densities and definitely indicated the need for complete overall spray application.

The number of spraying teams varied from two to three, each consisting of an American soldier acting as foreman and four Italian soldier laborers. At times each man operated his own Dobbins knapsack sprayer but usually two of the men were detailed to a knapsack sprayer having an eight foot extension spray rod (devised and provided by Malaria Demonstration Unit, Allied Commission). One man operated the pump, the other the extension rod. In this manner high ceilings were adequately treated.

It was of interest to note that in the bonifica which had been sprayed the previous season, the people welcomed back the spray crews with open arms; whereas in the areas to be treated for the first time, particularly the town of Castelvolturno, the people were quite hostile toward the temporary inconvenience of a disordered household. Once the people had experienced the benefits of DDT, however, they were continually asking for a repeat performance.

A summary of spraying activities, exclusive of the Mondragone AFHQ area, will be found in Table 4. A total of 1,383 houses required 1,269 pounds of DDT, which amounted to about 0.92 pounds per house. Application was made at the rate of approximately 83

Table 4—Summary of DDT Spraying Accomplishments in the Castelvolturno Area

	RURAL AREAS				URBAN AREAS	
	Bonifica di Castel-volturno (South)	Bonifica di Castel-volturno (North)	Bonifiche di Varcaturo and Lícola	Lago di Patria Qualiano Area	Castelvol-turno Town	Villa Literno Town
Gals. of DDT-Kerosene used	691	347	105	834	242	569
Pounds of DDT conc. used	314	158	48	380	110	259
No. of houses treated	255	146	36	313	275	313
Gals. of DDT-kerosene per house	6 (?)	* 6.25	6 (?)	6 (?)	* 5.5 (?)	* 16 (?)
Gals. of DDT kerosene used	2.71	2.37	2.64	2.7	0.9	1.8
Quarts of DDT per person	1.8 (?)	1.4	1.9 (?)	1.7 (?)	0.64	0.45
Grams of DDT conc. per person	93.1 (?)	74.4	100.8 (?)	93.9 (?)	23.3	23.5
Quarts of DDT per 100 sq. ft.	** 1.54 (?)	*** 1.63	** 1.66 (?)	?	?	?
Grams of DDT conc. per 1000 sq. ft.	79.7 (?)	84.1	86.4 (?)	?	?	?

* Population estimates, obtained locally, as follows: Castelvolturno—1500, Villa Literno—5000, Castelvolturno Bonifica—963 (actual count).

** Average interior wall space estimated to be 7000 sq. ft.

*** Three house types were involved, with the following interior wall space: Type I—4551.75 Sq. Ft. (99 houses), Type II—7922.75 Sq. Ft. (33 houses), Type III—9952.50 Sq. Ft. (14 houses).

milligrams of DDT per square foot. The differences in the amounts of DDT used in urban and rural areas may be explained by the smaller type of house in the towns.

At the start of this project, the opinion was expressed[1] that it was sometimes very difficult, particularly in a war torn area about to be occupied, to estimate DDT requirements on the basis of surface to be treated. It was suggested that estimates might be made on the basis of population. In the Castelvolturno project it was found that with an application rate of 83 milligrams per square foot, about two ounces (1.14 quarts of 5 percent solution) of DDT were required per person. Although population differences between rural and urban areas had a marked effect on the amount of DDT applied, it is felt, in the light of the highly satisfactory results obtained during the 1945 season, that reliable estimates might well be made on the basis of two quarts of 5 percent solution per person.

Entomological investigations — Inspections for hibernating mosquitoes by an entomologist from the 15th Medical Laboratory commenced 22 December 1944, and weekly visits to the Castelvolturno bonifica were maintained thereafter until the end of September 1945. Mosquito counts in general were made in an upstairs bedroom, a stable, and a pigsty at each station visited, using methods similar to those employed by the Malaria Demonstration Unit, Allied Commission, in 1944. Total mosquito counts were not made; rather, a specified area (preferred nesting place) in each room was inspected and counts were made accordingly. Total counts were made only in pigsties. Average wall and ceiling space inspected was as follows: Bedrooms — 23 square yards; stables — 27 square yards; pigsties — 14 square yards.

A total of twenty-three adult inspection stations and sixteen larval inspection stations were set up in the Castelvolturno bonifica and Qualiano areas (see map). In addition, during July and August, inspections were made in houses other than the regular stations, many being in that portion of the bonifica north of the river, and others were made in the Lícola and Varcaturo areas to the south. These varied in number from four to sixteen per week depending on how much time was available. All these houses had been sprayed. Mosquitoes were brought into the laboratory for identification, given the opportunity for oviposition, and were watched for signs of DDT intoxication.

In April it was decided that an area of untreated houses should be observed for comparison with the Castelvolturno project. The

[1] Personal communication from Dr. Henry W. Kumm, International Health Division, The Rockefeller Foundation.

Bonifica di Sessa was chosen (see map). It is a thinly populated area lying just south of the Garigliano River and the town of Minturno, consisting of the small village of Céllole (population approximately 3,500) and a few scattered farm houses. It was here after crossing the Volturno River at Cápua in October of 1943, that the Allied armies were held up for six long, dreary winter and spring months before they were able to force a break-through and join forces with the Anzio beachhead on the way to Rome. As a result of German destruction of the pumping station near the Garigliano River, the central portion of the bonifica was completely flooded, forming a marshy lake choked with vegetation about three and a half miles long and a half mile wide Fig. 2. In addition to the lake, anopheline larval habitats were to be found in numerous bomb craters, heavily overgrown canals and ditches and in a few small streams scattered throughout the area. Seven adult and seven larval inspection stations were established. Because of the tremendous numbers of mosquitoes present, inspections were placed on a semimonthly basis. Anyone who is not familiar with the habits of anophelines of the *maculipennis* group can hardly appreciate the tremendous populations which build up in an uncontrolled area. Because of their habit of invading houses and stables, inspections

Figure 2.—*Air view of the Sessa marsh during the* 1944 *season. By* 1945 *much of the lake expanse had become overgrown with reeds and other aquatic plants.* (Photo by J. M. Andrews).

for these anophelines are greatly simplified. In the Sessa Bonifica mosquitoes were so abundant that the walls and cobwebs were black with them and on being disturbed they sounded like a swarm of bees. In one pigsty it was estimated that there were easily a thousand mosquitoes per square yard of wall surface.

The first inspections made in the Castelvolturno area during December 1944 and January 1945 were negative, at least in the houses sprayed the previous season; other houses showed moderate numbers (as high as thirty-five) of hibernating adults. Mosquitoes brought into the laboratory at this time failed to feed or oviposit. Blood engorged anophelines were found in one pigsty on 28 December and 12 January. The first anopheline larvae (*Anopheles claviger* Meigen) were observed 19 March. Larvae (4th instar) belonging to one of the members of the *maculipennis* complex were not seen until 12 April. This latter observation, coupled with the fact that large numbers of males and females of *Anopheles maculipennis* (broadly speaking) were seen in the Sessa Bonifica on 28 April, suggests that oviposition probably commenced during the middle of March and the first brood appeared around the middle of April.

Anopheles claviger larvae were fairly common early in the year, but with the coming of warm weather its numbers fell off sharply. Of the *maculipennis* group, *Anopheles messeae* was the predominant species early in the season where ovipositions occurred. June collections showed a preponderance of *Anopheles labranchiae* but by August *Anopheles messeae* had resumed its former position. *Anopheles sacharovi* eggs were obtained on two occasions from the Castelvolturno area during the last week in May.

The results of adult mosquito inspections have been summarized in Tables 5 and 6. In the Castelvolturno area during June three stations had appreciable increases in adults far out of proportion to anything occurring in the remaining stations. Their monthly totals were as follows: Station III—45 adults, Station IV—154 adults, and Station V — 84 adults. The reason for the rise at Station III is not understood, because it dropped of its own accord in July and only 19 anophelines were found during the following three months. Investigations of Stations IV and V revealed two outdoor ovens and a wine cellar which had escaped the original spraying. After treating these (avoiding the remaining portions of the houses), adult members diminished but continued to prevail at a relatively low level. There were one or two other instances where it was necessary to return and spray an overlooked room or a formerly flooded outhouse,

but in each case the spraying was restricted to the room or outhouse in question. In general there was a slight buildup of the mosquito population about the middle of July but the numbers were insignificant compared with the Sessa comparison area, and judging from

Table 5.—Average Monthly Anopheline Adult Densities per Square Yard of Examining Surface — Castelvolturno Area*

Station	Area Examined Sq. Yds.	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sept.
C—I (B.P.S.)	75	0.00	0.00 (DDT)	0.00	0.00	0.00	0.03	0.01	0.01	0.01
C—II (B.P.S.)	49	0.42	0.12 (DDT)	"	"	"	0.13	0.03	0.01	0.06
C—III (B.P.S.)	95	0.14	0.05 (DDT)	"	0.01	0.01	0.09	0.01	0.01	0.03
C—IV (B.P.S.)	76	0.19	0.12 (DDT)	"	0.01	0.04	0.43	0.04	0.06	0.05
C—V (B.P.S.)	59	0.14	0.06 (DDT)	"	0.01	0.01	0.28	0.05	0.08	0.27
C—VI (B.P.S.)	40	0.00	0.00 (DDT)	"	0.00	0.00	0.00	0.15	0.19	0.15
C—VII (B.S.)	90	"	0.01 (DDT)	"	"	"	0.01	0.08	0.14	0.05
C—VIII (Pill Box)	16	"	0.00 (DDT)	"	"	"	0.00	0.00	0.00	Torn Down
C—IX (S).	22	"	0.00 (DDT)	"	"	"	0.09	0.02	0.06	0.02
C—X (B.S.)	80	"	0.04 (DDT)	"	"	"	0.01	0.00	0.00	0.01
C—XI (S.)	23	"	0.00 (DDT)	"	"	"	0.00	"	"	0.00
C—XII (B.S.)	48	"	0.00 (DDT)	"	"	0.02	0.04	0.03	0.01	0.06
C—XIII (P.S.)	32	"	0.06 (DDT)	"	"	0.00	0.00	0.01	0.00	0.03
C—XIV (B.S.)	33	"	0.00 (DDT)	"	"	0.01	0.01	0.02	0.00	0.01
C—XV (S.)	18	"	0.04 (DDT)	"	"	0.00	0.00	2.47 (DDT)	0.12	0.13
C—XX (B.P.S.)	48	———	0.00 (DDT)	"	"	"	0.01	0.00	0.00	0.00
C—XXI (B.P.S.)	48	———	"	0.00 (DDT)	"	"	0.00	"	"	"
C—XXII (B.P.S.)	48	———	"	0.00 (DDT)	"	"	0.01	"	"	"
C—XXIII (B.P.S.)	48	———	0.00 (DDT)	"	"	"	0.00	"	"	"
C—XXIV (B.P.S.)	48	———	0.00 (DDT)	"	"	"	0.01	"	0.01	"
C—XXV (B.P.S.)	48	———	"	0.00 (DDT)	"	"	0.00	"	0.00	"
C—XXVI (stair well)	8	———	" (DDT)	"	"	"	0.15	0.18	0.06	0.31
C—XXVII (B.P.S.)	48	———	"	0.00 (DDT)	"	"	0.01	0.01	0.05	0.03
Monthly Average		0.06	0.02	0.00	0.001	0.003	0.05	0.13	0.04	1.06

* For convenience, weekly collections have been combined with monthly totals.
** Station symbols: B—bedrooms, P—pigsty, S—stable.

Table 6.—Average Monthly Anopheline Adult Densities per Square Yard of Examining Surface — Bonifica di Sessa[1]

Station[2]		Area Examed Sq. Yds..	April	May	June	July	August	September
S—I	(G.S.)	14	1.00	17.86	35.71	145.85*	Discontinued	
S—II	(S.)	24	0.58	11.45	25.00	85.55*	50.00	50.00
S—III	(S.)	6	4.83	27.00	____	166.66*	116.66*	116.66*
S—IV	(S.)	19	1.11	4.53	13.16	78.94*	78.94*	78.94*
S—V	(S.)	71	1.73	14.08*	70.42***	_____	112.67*	112.67*
S—VI	(P.)	10	50.00	200.00*	1000.00***	_____	700.00*	700.00*
S—VII	(S.)	20	____	18.00	25.00	_____	55.00*	55.00*
Monthly Average			9.87	41.84	167.04	118.50	185.54	185.54

[1] For convenience, semimonthly inspections have been combined into monthly totals.
[2] Station symbols: G.S. — Goat shed, P. — Pigsty, S. — Stable.
*Based on estimate of over 1000 mosquitoes.

the spleen and parasite surveys conducted in August, they were too low to have an appreciable effect on malaria transmission.

As mentioned previously, additional checking stations other than the regular ones were visited at random during July and August. As expected, the numbers of adults recorded were in complete accord with the current situation existing elsewhere in the Castelvolturno Bonifica.

Average monthly densities (per square yard of preferred resting surface) of adult anophelines have been summarized at the bottom of Tables 5 and 6. The profound differences existing between the Castelvolturno and Sessa areas will readily be seen. Whereas in the Castelvolturno area monthly densities have been reduced to fractions of mosquitoes per square yard, in the Sessa area there are close to two hundred mosquitoes per square yard at the height of the season. During the entire seven and a half month period following DDT spraying a otal of 1,095 anophelines were captured in regular inspection stations in the Castelvolturno area; compared with this, in the Sessa Bonifica more anophelines were observed in a single catching station on one visit than were collected during the entire season at Castelvolturno .

The results of the larval surveys are to be found in Table 7. Because of the scarcity of adults in the Castelvolturno area, anopheline larvae became very difficult to find. Although larvae must have been distributed rather evenly throughout zones C, D, E, and F (Castelvolturno) as well as in the Varcaturo Bonifica, judging from the records of the adult stations their densities were extremely low. Elsewhere in the experimental area favorable aquatic situations were scarce or non-existent except in the bonifica north of the Volturno River. Even here, while there was an abundance of favorable situations, larvae were practically non-existent. On the other hand while not outstanding, collections in the Sessa Bonifica demon-

Table 7.—Average Monthly Anopheline Larval Densities per Ten Dips of Examination Surface —Castelvolturno and Sessa Areas

Station	Type	April	May	June	July	August	September
C—1	Canal	0.00	0.00	0.00	0.00	0.00	0.00
C—2	Flooded area	0.00	0.00	0.00	0.00	0.00	0.00
C—3	" "	0.00	3.00	0.00	0.00	0.00	0.00
C—4	Canal	0.00	0.00	0.20	0.00	0.00	0.00
C—5	"	0.75	0.00	0.00	0.00	0.00	0.00
C—6	"	0.00	0.00	0.00	0.00	0.00	0.00
C—7	"	0.00	0.00	Dry	Dry	Dry	Dry
C—8	"	0.00	0.00	3.50 (Dry)	Dry	Dry	Dry
C—9	"	0.00	Dry	Dry	Dry	Dry	Dry
C—10	Lake front	0.00	0.00	0.00	0.00	0.00	0.00
C—11	Canal	2.00	0.00	Dry	Dry	Dry	Dry
C—12	"	0.00	0.00	0.00	0.50	0.00	0.00
C—13	Ditch	0.00	0.00	0.00	0.00	0.20	0.00
C—14	Canal	0.00	Dry	Dry	Dry	Dry	Dry
C—15	"	0.00	0.00	0.00	0.00	0.00	0.00
C—16	"	0.00	0.00	0.20	0.00	0.00	0.00
Monthly Average		0.17	0.21	0.32	0.06	0.02	0.00
S—1	Swamp	0.00	2.00	21.00	0.00	11.00	———
S—2	Canal	0.00	0.00	0.00	0.00	0.00	———
S—3	"	0.00	0.00	Positive	6.00	0.00	———
S—4	"	2.00	10.00	35.00	Dry	Dry	———
S—5	"	5.00	20.00	22.00	0.00	10.00	———
S—6	Flood area	2.00	47.00	Abundant	0.00	0.00	———
S—7	Swamp	19.00	51.00	"	0.00	Dry	———
Monthly Average		4.00	18.60	11.60(+)	1.00	4.00	

strated reasonably large numbers of larvae. Because of the tremendous adult population it was anticipated that a greater number of larvae would have been found. It is presumed that the heavily vegetated marsh lake was the principal source of anophelines. If this is true it is quite possible, considering the extensiveness of the lake, for a large anopheline population to be present yet for the density of larvae in any one location to be low.

As originally planned no larvel control was to be permitted in the experimental area. Unfortunately a small amount of Paris green hand-casting was done by the Italians in a few isolated localities but after noting how carelessly it was done and how many places were overlooked it was felt that their work was inconsequential. The Allied Force Headquarters bathing beach section at Mondragone (eight square miles), however, was larvicided with oil in addition to being house sprayed but its location in the northwestern corner of the experimental area prevented it from having any considerable influence on the project.

In summary it can be said that there has been approximately a 97 and a 99 per cent reduction of anopheline larvae and adults. respectively, in the Castelvolturno area as compared with the Sessa area for the months of April, May, June, July, August and September.

Spleen and parasite surveys — As mentioned previously, three spleen and parasite surveys of the inhabitants of the Castelvolturno Bonifica (Zones A to F) were conducted by the Malaria Demonstration Unit, Allied Commission. during the course of their studies in 1944. Surveys were made in May, August and October. Only children between the ages of 5 and 15 years were examined. Spleen sizes were recorded after the mthod of Schüffnr.

At the beginning of the present project another survey was initiated in the same area in January. Two additional surveys were undertaken this season, in May and during the last two weeks of August. Experienced personnel were kindly loaned by the Malaria Demonstration Unit, Allied Commission, and slides were examined by technicians from the laboratory of Professor A. Missiroli, Instituto Superiore di Sanita, Rome.

The January survey, in addition to the Bonifica di Castelvolturno, included the town of Castelvolturno, which was DDT-sprayed for the first time this year but which had received a minor amount of pyrethrum spraying by the Italians the previous season. In May the survey was extended to include the Bonifica di Sessa comparison area and the town of Céllole. The results of these surveys are summarized in Tables 2 and 3.

As pointed out by Hackett (1937), spleen enlargement persisting in winter indicates *en masse* precisely what we wish to know, namely, the intensity of the malaria to which the population has been previously exposed."[2] In the Castelvolturno Bonifica at the start of the DDT-spraying operations there was a splenic index in May 1944 of 43 percent with the average spleen having a value of 1.6 corresponding to position P of the average spleen. This alone is not particularly significant but if one considers that there was a corresponding parasitic index of 21 percent one might presume that there had been a fair amount of transmission taking place the previous fall. By May of 1945, after one season's limited experience with DDT, the splenic index had dropped only one point (42 percent). The value of the average spleen had remained the same (1.6) but the parasitic index had dropped to 5 percent. It is unfortunate that these studies are being terminated before it is possible to make another survey this coming winter. Such a survey should provide conclusive proof that there had been greatly reduced malaria transmission as a result of large scale DDT operations completed early in 1945. The fact that there was a reduction in the parasitic index at the height of the transmission season from 12 percent (August 1944) to 1 percent (August 1945) and a previous high of 21 percent in May 1944 is indicative that malaria transmission was decidedly on the wane; coupled with this there was a definite shift to the left in the degree of splenomegaly, which suggests that malaria transmission in 1945 was practically nil. Unfortunately, information is not available to make a specific breakdown of positive smears except for the last survey made in August; of the six positive smears, one contained *Plasmodium falciparum* and five *P. vivax*.

While the indices in Castelvolturno town were in general lower than those in the bonifica, little information can be obtained from them as we have no means of comparing the two years' experience.

On the other hand the conditions found existing in the comparison area of the Sessa Bonifica and the town of Céllole are of extreme interest. In May of 1945 there was a splenic index of 56 percent, with the average spleen having a value of 1.9 and a parasitic index of 18 percent. By August the splenic index had risen to 63 percent, with an average spleen value of 2.2 — a definite trend to the right in degree of splenomegaly; the parasitic index was now 41 percent. Of the 87 positive smears examined, 30 (34 percent) contained *Plasmodium vivax*, 53 (61 percent) demonstrated *P. falciparum* and

[2] Hackett, L. W., "Malaria in Europe," Oxford University Press, London: Humphrey Milford (1937).

4 (5 percent) were mixed infections; gametocytes were found in 35 percent of the smears.

In addition certain statistics although incomplete are available from the local civilian health officer. Céllole has a population of approximately 3,500. During 1944 there were officially reported 822 cases of malaria between May and December. In 1945, from January through the middle of August, 1,058 cases came to official notice. In addition there were twenty deaths attributed to malignant tertian infections; in all cases parasites of *Plasmodium falciparum* were found. Considering these facts, the situation in the Bonifica di Sessa and Céllole town was in marked contrast to that existing at Castelvolturno. The high incidence of parasitism, together with the preponderance of *Plasmodium falciparum* infections and gametocyte carriers is indicative that malaria of epidemic proportions was present.

Antimalaria drugs were occasionally administered by local health officers in acute cases or by our own organization at the time surveys were being undertaken. In general, however, because of their scarcity the use of drugs, either as suppressive or therapeutic agents, has at no time played a significant part in the reduction of parasitic indices in any of the areas under observation.

Summary

Between January and April 1945 a single application of 5 percent DDT in kerosene was applied to the interiors of all houses and other shelters covering an area of approximately 93 square miles at the mouth of the Volturno River, near Naples, Italy. Interior surfaces were treated at the rate of about 83 milligrams of DDT per square foot. In terms of population this amounted to about 2 ounces (1.14 quarts of 5 percent solution) of DDT per person. Larviciding activities were carried on around the Mondragone bathing beach area (approximately eight square miles) in the northwestern corner of the experimental area. Elsewhere, however, no larviciding was accomplished except for a small amount of extremely localized and desultory Paris green handcasting by the local Italians.

Entomological investigations indicated the following species of anophelines to be present in the area: *Anopheles claviger, A. messeae, A. labranchiae,* and *A. sacharovi. Anopheles labranchiae* appeared to be the dominant anopheline during June and July. Weekly larval inspections were made at regular stations from December 1944 through September 1945; during July and August, additional treated stations were inspected at random. In April an untreated comparison area was established in the Sessa Bonifica, just to the north

and separated from the Volturno River basin by a range of hills. Because anophelines were so abundant inspections were placed on a semimonthly basis. There was approximately a 97 and 99 percent reduction in anopheline larvae and adults, respectively, in the Castelvolturno area as compared with the Sessa area for the months of April, May, June, July, August and September.

Spleen and parasitic surveys of children between the ages of 5 and 15 years were conducted in the Castelvolturno Bonifica over a period of a year and a half and in the Sessa Bonifica during the past year. In the Castelvolturno area a splenic index of 43 percent together with a parasitic index of 21 percent made during May 1944 is indicative of a considerable amount of transmission the previous autumn. By August of 1945 the splenic index had dropped to 25 percent and the parasitic index to 1 percent; together with this drop in the splenic index the degree of splenomegaly showed a decided shift to the left, suggesting that malaria transmission during 1945 had practically ceased.

On the other hand splenic indices in the Sessa Bonifica had increased from 56 percent to 63 percent between May and August 1945 and the degree of splenomegaly shifted to the right. The parasitic index had also moved from 18 per cent in May to 41 percent in August, and 61 percent of the infections were caused by *Plasmodium falciparum.* These statistics, together with the information from the local health officer to the effect that there had been 20 deaths due to malignant tertian infections in the area this summer indicate that malaria of epidemic proportions was present in the unprotected Sessa Bonifica during 1945.

Because of their scarcity antimalaria drugs were never in general use and it is felt that they played no significant role in the reduction of parasitic indices in any of the areas under observation.

EXTENT OF MALARIA RESEARCH IN THIS COUNTRY AND ABROAD

The Committee on Medical Research are making a survey of malaria research being carried on in this country and abroad. As space in the Journal permits and information becomes available we shall include as many of these items as possible. —The Eritor.

Emory University, Atlanta, Ga.

(Dr. W. B. Redmond)

An apparatus has been constructed to allow cultivation of plasmodia outside the host. It consists of two tubes, blood being circulated in one and a buffered nutrient medium in the other. A cellophane membrane interposed between the two tubes is permeable to the constituents of the nutrient medium but not to the blood proteins. Blood is circulated under pressure and the pH of the nutrient medium is kept under control. Malaria parasites grow readily in this apparatus and segmentation occurs, but very few of the merozoites enter new erythrocytes.

The parasite currently employed in these studies is *Plasmodium relictum.* Changes brought about in the blood by its metabolism correspond with those determined in the Warburg apparatus.

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(Dr. L. A. Terzian)

Recent studies have concerned reactions of the warm-blooded host to plasmodia. In particular, the effects of splenectomy prior to infection and during latency, using chicks infected with *Plasmodium lophurae* or *P. gallinaceum,* have been studied. The influence of oxygen at various pressures on the course of *P. gallinaceum* infection in chicks subjected to this gas in pure form has been investigated. Work has also been done on the effect of quinine in chicks infected with *gallinaceum,* from the standpoint of the pigmented and exo-erythrocytic cycles of the parasite.

PIGMENTATION AS A SPECIFIC CHARACTER IN CERTAIN ANOPHELINE PUPAE

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(Received for publication 15 April 1946)

During the course of the mosquito rearing work at the Columbia, S. C., insectary a probably undescribed pupal character has been observed to occur in laboratory reared specimens. This consists of the pigmentation of the pupae of certain anopheline species of mosquitoes.

Seven species have been observed and the pigmentation noted, as follows:

Anopheles quadrimaculatus (figs. 1 and 2) is the only species observed which possesses a distinct dark brown or black crescent-shaped marking between the two multiple hairs on the dorsum of the first abdominal segment. The marking is discrete and is apparently a pigmentation of the integument itself. In some pupae (probably, mostly males) this crescent is reduced to a thin line which may appear to extend dorsally over the mesonotum in two fine lines, ending in small squares of coloration.

A. m. freeborni (figs. 1 and 2) has a rather coarse diffused area of pigmentation on the mesonotum. This area forms an indiscrete "Y" with its apex at the base of the dorsum of the thorax.

The coloration is rather obscured, as though it were seen through, rather than being actually in the integument.

The apical margin of the first abdominal segment may be thickened and darkened although superfically resembling the smallest of the crescents of *A. quadrimaculatus;* however observation under a low powered glass will readily indicate the absence of definite pigmentation.

A. punctipennis (fig. 1) may be recognized by the paucity of pigmentation on the thorax and base of the abdomen, and particularly by the presence of two areas of black coloration on the dorsal abdominal surface. These areas are composed of "stipple-like" dots and do not have definite boundaries. The first area is on the second segment, while the second area is mainly on the fourth segment, but extends caudally into the fifth.

A. m. occidentalis has not been under observation in sufficient numbers to furnish positive data. However, it appears to have the "Y" of *A. m. freeborni* in a reduced condition, as well as the abdominal spots of *A. punctipennis*.

A. c. crucians, A. c. georgianus, and *A. walkeri* are without distinctive markings and must be separated from each other by characters not concerned with pigmentation. However, the lack of colored areas becomes in itself a character when compared to one of the pigmented species and is constantly used as such when but two species are concerned.

The above characters have been used with laboratory reared specimens over a period of three years with satisfactory results. Although borderline specimens do occur and coloration is difficult to observe in very new or very old pupae, separation of species by pupal coloration has been very helpful.

The possibility of the use of this character in the field seems plausible. However, darker coloration of field specimens may be a masking factor. In view of the differences exhibited between *A. quadrimaculatus* and *A. m. freeborni,* this type of character may be found helpful in separating members of the *maculipennis* complex.

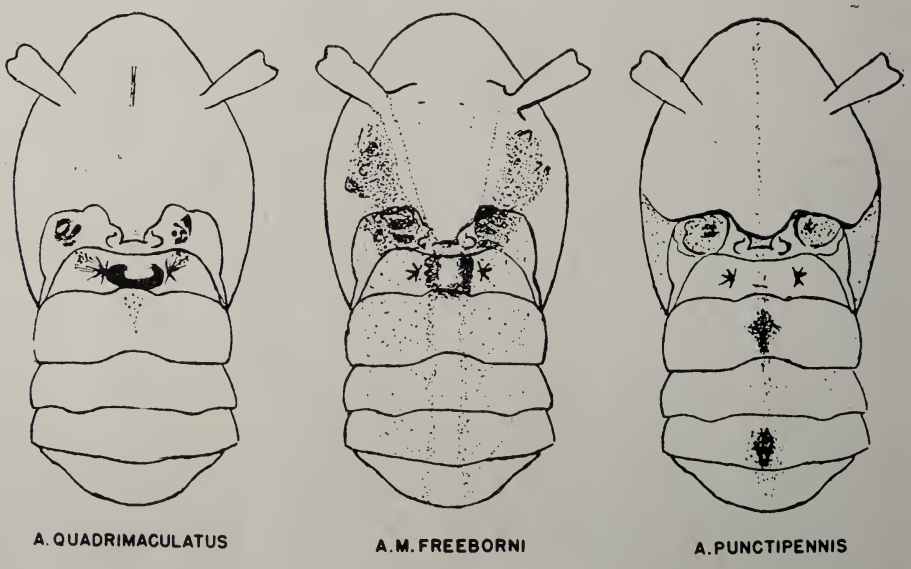

Figure 1. Diagramatic drawing showing pigmented areas in three species of *Anopheles.*

Figure 2. Photographs of *A. quadrimaculatus* and *A. m. freeborni* showing the natural appearance of pigmented areas.

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(Dr. N. H. Taliaferro; Dr. Clay G. Huff)

Studies on cellular and humoral factors in immunity, until recently carried on under O.S.R.D. contracts, are continuing with aid from private foundations. Histological studies on chicks infected with *Plasmodium gallinaceum* and *P. lophurae* are being carried on, to determine cellular reactions during infection and superinfection. The importance of the lymphocyte in immunity to plasmodial infections is being particularly investigated.

The relationship between immunity and quinine treatment is being studied with *P. gallinaceum* in chicks. The effect of immunity on the asexual cycle is being investigated in monkeys infected with *P. cynomolgi* and *P. knowlesi,* and in chicks infected with *P. gallinaceum* and *P. lophurae.*

Cellular aspects of immunity are being observed in chick embroys, and macrophage cultures have been employed to demonstrate opsonins in blood of hyperimmunized chicks.

Studies on the development of *P. gallinaceum* from sporozoite to erythrocytic trophozoite, and on the distribution of sporozoites and pre-erythrocytic stages in the chicken, carried out previously under O.S.R.D. contracts, are being followed by similar studies using *P. relictum, P. cathemerium* and *lophurae.* Searches for pre-erythrocytic stages of *P. elongatum, P. cynomolgi, logia,* and *P. vivax* are in progress.

Investigations are being carried out on the action of certain antimalarial drugs on the pre-erythrocytic stages of *P. gallinaceum,* and differences in behavior of pre-erythrocytic and erythrocytic stages of *P. gallinaceum* and *P. relictum* as regards natural and acquired immunity are being studied.

Continuation of these investigations in a variety of host animals, including embryos, is contemplated. Currently, a number of species of plasmodia are maintained in human, simian, and avian hosts, and sporozoites are grown for several species in the appropriate mosquitoes.

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Studies directed toward establishing the identity of different strains of plasmodia by the immunological approach are being conducted. An attempt is also being made to determine whether simian malarias might be utilized to produce immunity against human species. Comparison of immunity resulting from sporozoite infections and that produced by blood-induced infections is being carried out in human and avian malarias.

Investigations on the possible exo-erythrocytic cycle in human and simian malaria are being conducted. Drugs, both Indian and foreign, are being tested against simian and avian malarias.

Parasites maintained include *Plasmodium vivax, P. falciparum, P. knowlesi,* and *P. gallinaceum.* All except *knowlesi* are available as sporozoites.

A REVIEW OF MALARIA STUDIES AND CONTROL IN THE TENNESSEE VALLEY IN 1945*

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(Received for Publication 13 November 1945)

Introduction

The summer of 1945 was anticipated with some trepidation by those responsible for combating the hazard of malaria associated with impounded water projects of the Tennessee Valley. The last cyclical rise in malaria prevalence in the Tennessee Valley had occurred in 1938 and 1939. Epidemiological studies over a period of twenty years had indicated that such rises might be expected every five to six years. The incidence of malaria as determined by annual autumnal survey data revealed for the years 1943 and 1944 the lowest malaria rates on a Valley-wide basis ever recorded. In fact, only twenty positive blood films were found, of 15,338 examined, in these two years in the entire Valley, although only populations most likely to have malaria were survived. This number was often found in two or three houses in the epidemic year 1934.

Immunologically, therefore, the stage was set for a sharp increase in malaria in 1945. On the basis of survey data and the few confirmed cases of malaria during the summers of 1943 and 1944, it had to be assumed that gametocyte carriers were present throughout the Valley in 1945, probably to a much greater extent than could be shown by parasitological data. It had also to be assumed that to these indigenous carriers would be added others in the persons of returning service men with relapsing vivax malaria.

Our concern of the possibility for at least local outbreaks of malaria in epidemic proportions was enhanced by the fact that unusually warm weather in the spring resulted in initiation of active propagation of *Anopheles quadrimaculatus* fully a month earlier than ever before in our experience. Adults began to emerge from natural breeding places during the latter part of March. The plant growing season was also a month earlier than average and the growth of vegetation in the marginal zone of fluctuation in reservoirs was well advanced before the reservoirs could be filled to top summer pool levels. In Kentucky Reservoir, this circumstance was of particular concern for the reason that this lake had never before been

*Read before the twenty-eighth annual meeting of the National Malaria Society, Cincinnati, Ohio, November 12-15, 1945.

fully impounded. The flooding of the marginal vegetation, together with the prevalence of moderate temperatures and high humidities, created throughout the Valley almost ideal conditions for the propagation and survival of *A. quadrimaculatus*. In Kentucky lake, these conditions were optimum, for in addition to the new growth of marginal vegetation there was a further complicating factor of excessive flotage, a condition which is normally associated with the initial impoundage of a new reservoir and which invariably leads to heavy production of *A. quadrimaculatus* when it occurs during the mosquito breeding season. Moreover, unusually heavy spring rains throughout the Valley filled every natural depression with water, thus greatly increasing the production potential for *A. quadrimaculatus*.

As a result, there was an early uncontrolled and essentially uncontrollable propagation of *A. quadrimaculatus* throughout the lower two-thirds of the Valley. During June, on several main river reservoirs, adult station counts reached the highest level in our 12 years' observations, and in Kentucky mean station counts reached a peak of about 125 mosquitoes per station. When it became possible to institute favorable water level management schedules, this measure with the application of larvicides resulted in a fairly satisfactory control of mosquito production. Throughout most of the Valley, mosquito densities subsequent to 1 July were held at relatively normal levels. There were, however, areas in Guntersville, Wheeler and Kentucky Reservoirs, particularly the latter, where *A. quadrimaculatus* from impounded water breeding places maintained a mean density sufficient for malaria transmission throughout the entire season.

In spite of these circumstances, there is no evidence that there was a marked increase in malaria prevalence anywhere in the Valley during 1945. This fact is attributed to the low prevalence in the Valley at the beginning of the summer and to the measures which had been and which were instituted to prevent transmission. The extent to which transmission actually occurred, as measured by malaria survey data, cannot be defined until the blood films taken during the latter half of September have been examined, a task which will not be completed until midwinter.

Epidemiological Investigations

For the first time, an attempt was made to find and to treat malaria cases as soon as possible after they occurred. Case finding was done through enlisting the aid of all field workers associated with the malaria control program, regardless of their responsibilities.

The Biology and Physiology of Anopheles quadrimaculatus

The demands upon the mosquito inspection and identification service were considerably expanded during the 1945 season due to the initial impoundage of Kentucky Reservoir. Some 140 adult catching stations were established on this reservoir and were inspected at weekly intervals during the mosquito breeding season. Considerable information was obtained on the anopheline breeding potentials of flooded terrestrial plants. The average number of *A. quadrimaculatus larvae* per square foot for some representative species follow: wild millet, 49.9; lespedeza, 10.7; crab grass, 7.6; cocklebur, 6.8; giant ragweed, 6.4; broomsedge, 5.8. Entire areas were found to average as many as 6 larvae per square foot which is equivalent to over a quarter of a million per acre.

During the winter of 1944-1945, an outdoor insectory was established at Wilson Dam for studying the overwintering habits of *A. quadrimaculatus* under natural conditions. Studies in this outdoor insectory verified previous observations that hibernating *A. quadrimaculatus* adults emerge from hibernation during warm periods throughout the winter to take blood meals. Investigations are now under way to obtain further information on such questions as the latest and earliest dates of emergence and oviposition, gonotrophic dissociation, and overwintering of malaria parasites.

In the spring of 1945, an investigation was begun to determine the relation of various chemical factors to the production of *A. quadrimaculatus*. Studies were made of the surface water in breeding and nonbreeding areas to determine the relative contents of oxygen, carbon dioxide, hydrogen ions, nitrites, nitrates, ammonia, total organic nitrogen, and phosphates. In addition to the correlation of these chemical factors with larval densities, comparative data were collected on the quality and quantity of plankton and associated macroscopic organisms.

Work on the physiology of *A. quadrimaculatus* was accelerated. Basic information was obtained on the mechanism of the toxic action of DDT on insects. Further work on the physiology of the salivary glands of mosquitoes provided some promising clues as to the reasons for the relative roles of anophelines and culicines on the transmission of malaria and other diseases such as filariasis.

Insecticidal Studies and Demonstrations

A large-scale demonstration of DDT larvicide applied as a thermal aerosol, by airplane, was undertaken in partions of the Wheeler and Kentucky Reservoirs on the basis of previous experi-

mental studies (Metcalf, et al, 1945)[1]. The information reported here was taken from records of the demonstration in the Wheeler Reservoir since a more stable mosquito-breeding situation prevailed there than in the Kentucky Reservoir.

Two separate areas known as Buckeye Swamp and Edmondson Slough were treated. The application covered breeding areas within 1¼ miles of the center points and was on a routine basis at weekly intervals over a period of 16 weeks from 29 May to 11 September. The rate of treatment was approximately 0.12 pound of DDT per acre applied as a thermal aerosol in a 20 per cent solution of Velsicol NR-70[2] solvent on swath widths of 100 feet. The airplane used was one of the regular TVA 450 h.p. Stearman 4-DX dusters equipped also for thermal aerosol application. Significant improvements in the aerosol generators for this airplane were made through the use of an improved type of discharge nozzle and by shortening the recovery section of the Venturi. Detailed information was accumulated on the swath distribution and particle size composition of the aerosols produced by this generator. A total of 45 flying hours was required in treating some 16,000 cumulative acres of breeding area using 1300 gallons of the 20 per cent DDT solution. The total cost for the DDT treatment has been estimated at $0.35 per acre per application as compared with $0.85 for Paris green dusting under similar conditions. More effective larvae kills resulted than would have been obtained with Paris green dusting. Before and after treatment, dipping records indicated that a mean larvae reduction of at least 95 per cent may be anticipated. As in the case of Paris green dusting, it was necessary to limit the applications to the early morning hours and there were the usual interruptions and postponements caused by wind and rain.

In addition to the lower unit cost, DDT is a much cleaner and easier larvicide to mix and apply than Paris green. A 50-gallon tank of the DDT larvicide weighing 470 pounds would treat nearly 1000 acres, while a full hopper of 22 per cent Paris green dust weighing 1000 pounds would treat only 220 acres at the one pound per acre rate of application.

The Authority is now considering the matter of using DDT in place of Paris green for larvicidal treatment by airplane. The conversion would offer no equipment problem.

[1] Metcalf, R. L., A. D. Hess, G. E. Smith, G. M. Jeffery, and G. W. Ludwig. Observations on the use of DDT for the control of *Anopheles quadrimaculatus*. Public Health Reports, 60:753-774, 1945.

[2] A polymethyl naphthalene consisting mostly of siomeric tetramethyl napthalenes.

Mosquito station inspectors and local residents who had been trained in public health education were especially helpful. They reported to health officers through appropriate channels the occurrence of illness which might conceivably be due to malaria infection. Subsequently these reports were investigated by public health nurses or by a physician. At these visits, blood films were taken not only of the person who was supposed to have malaria but also of all members of the household and sometimes of adjacent households. In addition, in April and May, resurveys were made of the families of 13 persons having positive blood films in the 1944 Valley-wide autumn survey, and of families adjacent thereto. Provision was made for the treatment of confirmed cases of malaria by health agencies in Alabama and Tennessee.

The spring resurveys found only two persons whose blood films contained demonstrable malaria parasites, and these persons had negative blood films in the previous autumn. Among the reported cases of malaria investigated during the summer of 1945, only five positive blood films were found. One of these was in Wheeler Reservoir, one in Guntersville Reservoir, and three in Kentucky Reservoir. Two of the three cases in Kentucky Reservoir were in the same general area but outside the mile zone of the lake. One of these cases may have been secondary to a case of relapsing malaria in a returned service man.

It is our belief that the very small number of confirmed cases of malaria found underestimates the total number of cases of malaria which occurred in the Valley during 1945. In every instance a person who may have had malaria had taken more or less adequate treatment before a blood film could be secured. Nevertheless, the total number of reported cases of malaria which conceivably might have been due to malaria was not as high as might have been expected on the basis of the prevalence of anophelism.

The Relation of Plants to Malaria Control

The plant growth invasion plots which were established in the spring of 1944 were expanded to include all of the nine main river reservoirs and six of the more important storage reservoirs. Readings from these plots were used to make current decisions concerning water level management; for establishing correlations between water control schedules and the rate and extent of invasion of marginal plants; and for determining the effect of water level schedules upon the nature and extent of the annual growth removal operations.

Other observations on the water level relationships of marginal

plants were continued during the 1945 season. Of particular importance was information on the effect of spring inundation in preventing germination or sprouting and the relative tolerances of different species to flooding. Summer flooding proved to be an effective means of controlling terrestrial species during their early stages of development, but it was relatively ineffective in controlling typical wetland species. This information emphasizes the importance of maintaining top summer pool level during the spring growth period in order to prevent plant growth in the zone of fluctuation, and thus provide a clean shoreline during the summer months.

Investigations were made on the extent of regrowth of various types of vegetation out at different periods in the fall. The results indicated that, in general, mowing operations in the Tennessee Valley should not be initiated before 15 September or, in some instances, until 1 October. However, some plants such as ragweeds, horseweed, and cocklebur were killed by summertime mowing. Studies on the overwintering of various herbaceous plants gave further evidence that flexuous species, such as grasses and smartweeds, and certain emergent aquatics, such as lizard's tail, almost completely disintegrate during the winter months. Decreased emphasis is therefore being placed upon the removal of the rough of these species during the annual shoreline conditioning operations, and primary importance is being given to the removal of stiff-stemmed terrestrials and the coppice of water tolerant woody species.

Observations on the results of shoreline grazing by livestock verified previous beliefs that this is a very effective method of marginal growth control. There were, however, a number of plants which were not commonly grazed, including smartweeds, lizard's tail, rushes, cocklebur, and stinkweed.

The use of herbicides for controlling the infestation of alligator weed on Wilson Reservoir continued to be unusually successful, and it is now difficult to find any sizeable colonies of this plant around the shores of the lake. Studies were initiated to determine the effectiveness of the new hormone-type weed killers, such as 2,4-dichlorophenoxyacetic acid, in controlling objectionable plant species. Results indicate that these materials applied in dosages as low as a pound or less per acre will control willow coppice and many dicotyledonous herbs. The use of "2,4-D" and related materials therefore appears to offer a new approach to the management and control of marginal plants. The low dosages in which they are effective make their application by airplane a definite possibility.

In cooperation with the Forestry Relations Department of the TVA, investigations were continued during the 1945 season to determine the effect upon fish and other aquatic organisms of DDT thermal aerosols applied by airplane in the manner described above. Although a complete analysis of the data has not yet been made, a preliminary appraisal indicates that DDT thermal aerosols applied at dosages of about 0.1 pound per acre will not result in any significant injury to wildlife. Forms which appeared to be particularly susceptible to the DDT thermal aerosols included some adult Diptera (Dolichopodiae, Culicidae, etc.) and certain types of neuston (Gerridae, Hydrometridae, Mesoveliidae, etc.). Forms which did not appear to be significantly affected included plankton, nekton (Zygoptera, Corixidae, Dytiscidae, etc.) and benthos (Chironomidae, Anisoptera, etc.). Two bays containing a wide variety of fishes were dammed off from a main river reservoir and treated with DDT thermal aerosols at weekly intervals from 29 May to 11 September at a rate of about 0.12 pound DDT per acre. During the latter part of September these bays were treated with rotenone in order to study the fish populations. A qualitative and quantitative appraisal of these fish populations indicated that the treatments were in no way harmful to the fish life.

Airplane dusting has largely replaced the application of larvicides by boat on Authority reservoirs, but a limited amount of boat work is expected to be required for some time. A kerosene-black oil mixture has been the larvicide in use. Experiments indicate that the boat and knapsack equipment could be easily converted for use of DDT as a larvicide. One of several solvents might be used, applied by the "water-oil"' method or as a very fine spray, with an increase in effectiveness and economy.

During the past season the Authority outfitted thirteen 200 h.p. Stearman PT-17 airplanes for application of larvicide for other agencies. Eleven of the airplanes were for applying DDT as a thermal aerosol, one was for dusting, and one was for applying DDT either as a spray or as a thermal aerosol. The actual shop work was done on a reimbursable service basis but Authority personnel devoted a considerable amount of time designing, developing shop drawings, and testing equipment covering the installations, and training pilots for the work.

Mosquito-Proofing and Residual House Spraying with DDT

Before 1 May 1945, new mosquito-proofing was applied to approximately 1150 houses in Kentucky Reservoir area, and to 200 houses in Guntersville Reservoir. This work was done in areas

most likely to be exposed to intense anophelism. Also, maintenance work (which included some new mosquito-proofing) was done in areas previously mosquito-proofed. In many instances, mosquito-proofing protection was fortified by DDT residual spraying.

With an allotment of DDT from the Malaria Control in War Areas' program of the U. S. Public Health Service, a large-scale demonstration of residual house spraying was done in selected areas bordering the Kentucky, Wheeler, and Guntersville Reservoirs during the 1945 season. During May a total of 3150 houses were given one treatment at the rate of 200 milligrams per square foot. A relatively small number of houses were treated during the latter part of the season at one-half this rate. The spray used was a 5 per cent water emulsion of DDT made from a concentrate consisting of 30 per cent DDT, 68 per cent xylene,[3] and 2 per cent Triton X-100 (emulsified). The work provided an opportunity to test various types of spraying equipment, develop techniques, and accumulate data for planning and estimating such work in the future. While most of the house spraying was done as a supplement to other measures, a portion of the work in the Kentucky Reservoir was done on a service basis for Malaria Control in War Areas and its extended malaria control program.

The cost of this program averaged $3.00 per house sprayed, allowing $0.60 per pound for DDT, $0.55 per gallon for solvent, $1.00 per pound for emulsifier, $0.65 per hour for sprayment, and 6½ cents per mile for truck travel. The total cost was, therefore, approximately 26 per cent for material, 64 per cent for labor, and 10 per cent for travel. A crew of two men averaged only about eight hours per day; considerable time was necessarily consumed in travel from the base to the point of work and in preparing the houses for spraying by covering and moving furniture.

The treatments appeared to be reasonably effective to the end of the mosquito breeding season. Precipitin tests with engorged *Anopheles quadrimaculatus* adults collected from barns and other structures adjacent to the treated dwellings indicated a significant reduction in the percentage of mosquitoes which had taken blood meals from humans.

During the past season, approximately 40 new organic compounds were tested for their toxicity to larvae and adults of the malaria mosquito, *A. quadrimaculatus*. Of these, the most promising were gammexane (the gamma isomer of benzene hexachloride) and pyrethrins. Toxicity tests indicated that gammexane is three

[3] Sovasol 74 manufactured by Socony-Vacuum Company.

to five times as toxic as DDT to *A. quadrimaculatus* adults. It was also highly effective as an anopheline larvicide. Pyrethrins exhibited remarkable residual toxicities for *A. quadrimaculatus* adults and gave much more rapid knockdowns than DDT; however, the per cent mortality obtained was usually somewhat lower. Two other materials which showed considerable toxicity to *A. quadrimaculatus* were dichlorodiphenyldichloroethane (DDT) and dibromodiphenyltrichloroethane.

Permanent Shoreline Improvement

The successful use of permanent shoreline improvement in the recently impounded Kentucky Reservoir gave impetus to the study of such possibilities in five other reservoirs of the lower valley. The preliminary studies indicated that the permanent approaches of diking and dewatering, and deepening and filling, can be applied to provide more adequate malaria control protection as well as the reduction of recurring costs. Deepening and filling is expected to receive an increasing use due to the almost negligible maintenance costs, and to the fact that water level management is limited to the marginal growth invasion to a depth of only two feet as a maximum.

Use of marginal lands for grazing has been employed widely on Authority land bordering the reservoirs. On gently sloping shoreline of not very fertile soil the incidental reduction of marginal growth in the lake has been significant. The conditions in one section of the Wheeler Reservoir are such as to warrant the systematic use of grazing for marginal growth control as a permanent approach in eliminating the repetitive use of larvicides. The plan anticipates some limited mechanical or herbicidal control of plants which are not grazed, some minor alterations of topography and fencing to provide both upland and lake pastures. Cattle will be alternated to advantage between the pastures.

Advanced planning is under way as an expansion of the permanent shoreline improvement program and some construction is expected to be undertaken during next year. Additional topographical data are needed for the advanced planning, and are being obtained in a unique way by successive aerial pictures taken at different lake levels. The water lines are easily identified, and excellent composite contour maps are being prepared, using the special mapping equipment of the Authority's Maps and Surveys Division. The necessary data are being obtained in this way for only a fraction of the cost and number of personnel required by the conventional plane table procedure.

In anticipating construction in reservoirs which have been im-

pounded, special earth-moving equipment is being investigated, such as hydraulic dredges and cable-operated drag scrapers. A place for such equipment may develop where boggy ground conditions will not support conventional land machines.

Minor Modifications of Shoreline Topography

The well-known bulldozer is being utilized in the development of methods for minor modifications of shoreline topography aimed at reducing the anopheline potential and the cost of applying repetitive measures. In one area a combination operation of leveling drainage ditch spoil banks and removing dead stumps and marginal growth of all types has been performed with a bulldozer. The cost was comparable to that for one year's growth removal operation by hand, in the same area. In treated areas, subsequent marginal growth can be removed by mowing machine at a fraction of the cost of hand removal operations used formerly.

Summary

The summer of 1945 presented one of the most interesting and potentially dangerous malaria control problems in the 12 years' experience of the Tennessee Valley Authority's malaria studies and control program. An increase in malaria prevalence was anticipated in conformance with past experience of cyclical increases in prevalence every five to six years. Temperatures suitable for the propagation and survival of *Anopheles* and for initiation of the growth of vegetation occurred fully a month earlier than normal. The newly impounded Kentucky lake and other lakes in the lower Valley were not filled to maximum summertime elevations until the growing season was well under way and had been extensively invaded by marginal plants. When the lakes were subsequently filled, almost optimum conditions for *A. quadrimaculatus* propagation resulted, particularly in Kentucky lake, and the highest adult densities ever observed by us occurred in several reservoirs.

When it became possible to establish appropriate water level management schedules, this measure together with application of larvicides succeeded in reducing propagation to approximately normal levels. However, sufficient densities (of the order of 25 mosquitoes per station per week) to permit transmission of malaria were experienced in many places in the lower Valley throughout the entire summer.

Field studies on malaria morbidity carried out throughout the summer failed to reveal evidence of a significant increase in malaria prevalence. While results from the autumn Valley-wide surveys are not yet available, it is not believed that data from them will materially change this finding.

It is believed that malaria transmission was kept in check by the low level of gametocyte carriers at the beginning of the season and by the application of preventive measures in critical situations. These consisted of a supplementation of the conventional antilarval program by mosquito-proofing and the application of DDT residual house spraying. The latter measure was done at the rate of 200 milligrams of DDT per square foot of wall surface in June and early July and appeared to be effective throughout the season.

Biological investigations included further observations on the relation of plants to malaria control with particular reference to the water level relationship of plants, the removal of obnoxious species by grazing, and the use of herbicides to destroy plants. The study of the biology and physiology of *A. quadrimaculatus* was continued in the field and in outdoor and indoor insectaries. These included ecological studies on the larval habitat, the habits of hibernating adults, the mechanism of the toxic action of DDT on adults and larvae, and further studies on the physiology of the salivary glands.

In cooperation with the Forestry Relations Department of the TVA, investigations were continued during the 1945 season to determine the effect upon fish and other aquatic organisms of DDT thermal aerosols applied by airplane in the manner described above. Although a complete analysis of the data has not yet been made, a preliminary appraisal indicates that DDT thermal aerosols applied at dosages of about 0.1 pound per acre will not result in any significant injury to wildlife.

Mosquito control operations of an investigative character include large-scale demonstrations of the application of DDT larvicide by airplane as a thermal aerosol, residual house spraying with DDT and other insecticides, and the large-scale use of permanent shoreline improvement measures.

In connection with the application of diking and dewatering and deepening and filling in impounded reservoirs, topographical studies were facilitated by the use of aerial photography. Use of bulldozers for minor shoreline topographical alteration and of hydraulic dredges and cable-operated drag scrapers for larger-scaled engineering works were investigated and promise to fill a definite place of usefulness in future operations.

TRAINING AIDS FOR ENTOMOLOGICAL PHASES OF MALARIOLOGY[1]

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(Received for publication 14 November 1945)

Current and anticipated problems related to malaria in the United States necessitates that a large number of individuals be trained in entomological phases of malariology. It is likely that an increase in the number and scope of academic courses pertaining to medical entomology and sanitary engineering, and the number of students taking those courses, will be one result of wartime interest in insect-borne diseases. There is a need to provide adequate training in basic principles of malaria for workers in public health. The probability of the introduction of malaria as a result of war travel creates the possibility of malaria problems in areas now free of the disease as well as in endemic areas. In many instances individuals who will be given such teaching assignments will not be thoroughly familiar with the subject nor the sources of recently developed teaching materials.

The Committee on Entomology believes that an outline of readily available illustrative and audio-visual material on *Anopheles* mosquitoes pertaining to malaria will be useful in planning the classroom and laboratory portions of the course. It will serve also to prepare students for field work; this is an essential part of a good malaria course. In some instances, supplemental information from the literature is necessary to achieve continuity. Many pertinent references are included in the listing. Information considered desirable, but not essential in a brief course of instruction, is listed as collateral material. So far as is possible the items are listed in the order in which they are presented most commonly.

No attempt has been made to include all the well-known and excellent works which provided a background for much of the material. Literature cited can be distributed to individuals because it is accessible and inexpensive.

[1] Opinions regarding the material for this report were solicited from several entomologists who are engaged in malaria activities. The Committee is indebted to Dr. Herbert Knutson for suggesting that this subject be presented, and for his helpful suggestions and criticisms relative to preparation of the report.

Report of the Committee on Entomology

The list of training materials is not intended to be exhaustive. The items listed are not necessarily believed to be the best available, but they can be obtained easily, and are broad in scope. With these teaching aids, lectures can be reduced to a minimum and may be prepared by using the collateral materials as a basis. Courses lasting from one to four weeks may be projected with the material listed. The amount of material used will depend upon the time allotted to lectures, laboratory, and field studies.

Taxonomic Position and Identification of United States Anopheles

Pictoral Charts—8 x 10½ inches:

1. *Pictorial Chart Differentiating between Anophelines and Culicines.
2. *Pictorial Key to Adult Female Anophelines of the United States.
3. *Wing and Mouth Parts of Female *Anopheles* of the United States.
4. *Pictorial Key to Anopheline Larvae of the United States.
5. *A Key to the Mosquito Larvae of Southern United States.
6. *A Key to the Mosquito Larvae of the Pacific Coast.

Film Slides (film strips):

1. *Preliminary Steps to Adult Mosquito Identification. — 35 mm., color, sound.
2. *Identification of Female Anophelines of the United States. — 35 mm., color, sound.
3. *The Genera of Mosquito Larvae of the United States. —35 mm., color, sound.
4. *Identification of Anopheline Larvae of the United States.—35 mm., color, sound.
5. *General Inspection and Control Activities. — 35mm., black and white, silent. Frames 2-5, 15-19.

Collateral Material:

1. Aitken, T. H. G. Studies on the Anopheline Complex of Western America, Univ. of California Publications in Entomology. 1945. 7:11, 273-264.
2. Carpenter, S. J., Middlekauf, W. W., and Chamberlain, R. W. The mosquitoes of the Southern United States, East of Texas and Oklahoma. 1946. American Midland Naturalist Monograph No. 3 Notre Dame University, South Bend, Indiana.
3. King, W. V., Bradley, G. H., and McNeel, T. E. The Mosquitoes of the Southeastern States. 1944. U. S. Department of Agriculture., Misc. Pub. 336.
4. Matheson, R. Hand book of the Mosquitoes of North America. 1944. Comstock Publishing Co., Ithaca, N. Y., pp. 3-29, 89, 95-116.
5. Naval Medical School. Arthropods of Medical Importance. 1944. National Naval Medical Center, Bethesda, Md.
6. Ross, E. S., and Roberts, H. R. Mosquito Atlas, Part 1. 1943. American Entomological Society, Philadelphia.

* These items may be obtained from the Office of Malaria Control in War Areas, U. S. Public Health Service, 605 Volunteer Building, Atlanta, Georgia.

7. Russell, P. F., Rozeboom, L. C., and Stone, A. Keys to the Anopheline Mosquitoes of the World. 1943. American Entomological Society, Philadelphia.
8. *U. S. Public Health Service. Identification Guide to the Mosquitoes of the Pacific Coast States. 1943. Office of Malaria Control in War Areas, Atlanta.
9. *U. S. Public Health Service, Entomological Field Handbook, Office of Malaria Control in War Areas, Atlanta, Ga.

Distribution and Biology of United States Onophelines

Pictorial Charts — 8 x 10½ inches:
1. *Distribution of Three Principal Malaria Vectors.
2. *Distribution of Nearctic *Maculipennis* Complex.

Film Slide (film strip):
1. *General Inspection and Control Activities. — 35 mm., black and white. Frames 24-32.

Literature:
1. *Entomological Field Handbook, op. cit. pp. 12-21.
2. Arthropods of Medical Importance, op. ct. pp. 96-98.

Motion Picture:
1. Mosquitoes—U. S. Department of Agriculture, 16 mm., black and white, sound.

Collateral Material:
1. Handbook of the Mosquitoes of North America, op. cit. pp. 30-46.
2. Studies on the Anopheline Complex of Western America, op. cit.
3. The Mosquitoes of the Southeastern States, op. cit. pp. 5-8, 29-39.
4. Identification Guide to the Mosquitoes of the Pacific Coast States, op. cit.
5. The Mosquitoes of the Southern United States, East of Texas and Oklahoma, op. cit.
6. Needham, J. G., and Needham, P. R. A Guide to the Study of Fresh Water Biology. 1941. Comstock Publishing Co., Ithaca, N. Y.

Anopheline Surveys

Motion Picture:
1. **Anopheles* Census — 16 mm., black and white, sound.

Film Slide (film strip):
1. *General Inspection and Control Activities — 35 mm., black and white. Frames 6-14, 20-44.

Literature:
1. *Entomological Field Handbook, op. cit., pp. 30-43.
2. Arthropods of Medical Importance, op. cit., pp. 103-111, 157-178.

New York University, Goldwater Memorial Hospital Welfare Island, New York

(Dr. David P. Earle — Dr. Robert Berliner)

Testng of drugs, and studying the action of anti-malarial agents, are the principal objectives of a new research program. *Plasmodium falciparum* will be used, and a technique has now been worked out for carrying out the studies by in-vitro methods. This parasite will be carried (Costa strain) in patients in sufficient quantities to provide material for the in-vitro work.

School of Medicine, University of Texas, Galveston

(Dr. Wendell Gingrich)

Several lines of investigation are being pursued, using *Plasmodium cathemerium* maintained in canaries. *Culex quinquefasciatus,* reared in the laboratory, are employed to furnish sporozoite infections.

Studies are directed toward immunology, including superinfection and laboratory diagnostic tests, and toward the exo-erythrocytic cycle and the problem of relapse.

The effects of various drugs against different stages of the infection are also being investigated.

Tennessee Valley Authority, Wilson Dam, Alabama

(Dr. E. Harold Hinman)

Investigations at present are chiefly of an epidemiological nature, and are directed toward determining the presence of malaria in areas around the Authority's reservoirs, by blood examinations and by morbidity reports. The efficacy of DDT residual spraying is being tested by several means, including an attempt at measuring the degree of malaria in treated and untreated areas, although "it is recognized that the present low endemicity of malaria may make this of no value."

It is planned to conduct experimental work in the near future on the insect-parasite relationship, probably using avian malaria.

The Public Health Research Institute of the City of New York

(Dr. Jules Freund)

Immunization studies have been carried on in monkeys and ducks, using *Plasmodium knowlesi* and *P. lophurae* respectively. These investigations are based on previous work of Freund, utilizing antigens incorporated in a lanolin-like substance which promotes slow absorption.

STUDIES ON SEROLOGY OF MALARIA[1]

II. *Hemoglobin Precipitation With Lipid Antigen**

Albert H. Wheeler, Elizabeth B. McDermott, Jacob Adler and Reuben L. Kahn

Clinical Laboratories, University Hospital, University of Michigan, Ann Arbor

(Received for Publication 4 March 1946)

It was observed in this laboratory that precipitation occurs on mixing hemoglobin solution (laked erythrocytes) with lipid (Kahn) antigen[1]. This observation was made in connection with studies on differentiation between hemoglobin solutions prepared from erythrocytes obtained from malarial (vivax) and nonmalarial persons. Hemoglobin solutions from erythrocytes of malarial origin were found to possess a greater tendency toward precipitation with lipid antigen than hemoglobin solutions of nonmalarial origin. Studies were thereupon undertaken of factors affecting this precipitation phenomenon. These studies are presented in this article. The factors include: 1. The ageing of the blood clots from which the hemoblogin is prepared. 2. The concentration of NaCl solutions used in the preparation of the hemoglobin solutions and of the lipid antigen suspensions. 3. The physical state of the lipid antigen suspension. 4. The hydrogen ion concentration of the hemoglobin solution. 5. Solubility of precipitates in excess of lipid antigen or hemoglobin solution. 6. Shaking of the hemoglobin solution. Precipitation reactions with hemin, globin, ferric chloride and potassium chloride with lipid antigen suspensions were also studied.

Hemoglobin Precipitation Reaction — Method

Preparation of hemoglobin solution. Clots from whole blood specimens are broken up mechanically in about 5 cc. of physiologic salt solution. Unbroken clumps remaining are removed by filtration through gauze. The resulting red cell suspension is washed free from serum as in the preparation of red cells for complement fixation tests. Four washings with saline are usually sufficient. The washed cells are packed in a calibrated centrifuge tube by centrifugation for 15 minutes at 1,800 rpm, the volume of the packed cells recorded, and the water-clear supernatant fluid discarded. The tubes are allowed to stand for 10 minutes and the residual supernatant fluid is removed as completely as possible. Distilled water is added to the packed

[1] The first article of this series was published under the title: Phenomenon of Precipitation on Mixing Hemoglobin With Tissue Extract Antigen, by Reuben L. Kahn, Elizabeth B. McDermott, Albert H. Wheeler and Jacob Adler, 1945. Proceed. Soc. for Exper. Biol. and Med. 58: 240-242.

*The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Regents of the University of Michigan.

cells to make a 1:5 dilution and the cells are hemolyzed as thoroughly as possible by stirring intermittently with a wooden applicator during a 10 minute period. The resulting turbid solution is centirfuged for 15 minutes at 1,800 rpm. The supernatant hemoglobin solution is removed carefully from the sediment with a bulb pipette and is employed in preparing serial dilutions of the desired range with distilled water.

Preparaton of lipid antigen suspension. Standard Kahn antigen was used in this study as the lipid antigen and the suspension was prepared by mixing the antigen, not with 0.9 percent sodium chloride solution but with 0.3 per cent. The amounts of the antigen and of the salt solution employed were based on the titer of the antigen with 0.3 percent salt solution. The method of mixing the antigen with the weak salt solution in preparation of the antigen suspension was the same as that employed in preparing the antigen suspension for the Kahn test.

Performance of test. In illustrating zones of precipitation in mixtures of hemoglobin solution and antigen suspension, serial dilutions of hemoglobin solution with distilled water are first prepared ranging from 1:5 to 1:1920. Each of these dilutions is then mixed with antigen suspension in the following ratios, 1:4, 1:2, 1:1, 3:1, 6:1, 12:1, 18:1, 24:1 and 30:1. The actual amounts of hemoglobin solution and antigen suspension employed are given in Table 1. The mixtures are shaken for a few seconds to assure proper mixing and the precipitation results read. Marked precipitation is recorded as 4 plus, precipitation in decreasing scale is recorded as 3 plus, 2 plus and 1 plus.

Table 1—Illustration of Zones of Precipitation Shown by Hemoglobin With Tissue Extract Antigen

Dilution of hemoglobin with water	Hemoglobin dilution, cc								
	0.05	0.05	0.1	0.15	0.15	0.15	0.18	0.24	0.30
	Antigen suspension, cc								
	0.2	0.1	0.1	0.05	0.025	0.0125	0.01	0.01	0.01
	Ratios of hemoglobin dilution to antigen suspension								
	1:4	1:2	1:1	3:1	6:1	12:1	18:1	24:1	30:1
	Hemoglobin 1								
1:5	4*	4	–	–	–	–	–	–	–
1:10	4	4	4	2	–	–	–	–	–
1:15	4	4	4	4	–	–	–	–	–
1:30	4	4	4	4	2	–	–	–	–
1:60	4	4	4	4	4	4	2	–	–
1:120	–	3	4	4	4	4	4	2	–
1:240	–	2	4	4	4	4	4	4	–
1:480	–	–	–	4	4	4	4	3	–
1:960	–	–	–	–	–	3	4	–	–
1:1920	–	–	–	–	–	–	–	–	–
	Hemoglobin 2								
1:5	4	4	4	4	–	–	–	–	–
1:10	4	4	4	4	4	2	–	–	–
1:15	4	4	4	4	4	4	3	2	–
1:30	4	4	4	4	4	4	4	4	–
1:60	4	4	4	4	4	4	4	4	1
1:120	–	–	4	4	4	4	4	4	4
1:240	–	–	2	4	4	4	4	4	2
1:480	–	–	–	2	4	4	4	1	–
1:960	–	–	–	–	–	4	2	–	–
1:1920	–	–	–	–	–	–	–	–	–

*The numbers 4, 3, 2 and 1 represent precipitation in decreasing scale.

Illustration of Zones of Precipitation in Mixtures of Hemoglobin Solution with Lipid Antigen Suspension

Table 1 gives an illustration of the precipitation reactions noted on mixing various ratios of hemoglobin solution with antigen suspension. The results of two different hemoglobin solutions are present in the table. In the 1:4, 1:2, 1:1 and 3:1 ratios of hemoglobin solution to antigen suspension, both hemoglobin solutions give approximately the same precipitation results. In the ratios of hemoglobin solution to antigen suspension extending from 6:1 to 30:1 hemoglobin solution 1 shows a lesser tendency toward precipitation than hemoglobin solution 2. The increased tendency toward precipitation was more frequently noted with hemoglobin solutions prepared from malarial blood specimens. It was believed essential, however, to first study the nature of this precipitation reaction before attempting to investigate its possibilities in the practical differentiation between malarial and nonmalarial blood specimens.

1. *Effect of Ageing of Blood Clots in Serum and in Saline*

Preliminary experiments indicated that the ageing of blood clots in serum for various lengths of time at room temperature tended to increase the precipitability with lipid antigen of the hemoglobin solutions prepared from these clots. The present experiment was designed to determine, first, whether the same effect would be exerted if the serum were replaced by 0.85 percent saline before such ageing, and second, if malarial and nonmalarial blood specimens would give the same results after ageing.

The clots from 37 malarial and 20 nonmalarial blood specimens were split into two parts, one of which was left in a given amount of serum while the other was placed in an amount of 0.85 percent NaCl solution equivalent to the amount of serum. These were incubated overnight at room temperature and then used for preparing hemoglobin solutions in the regular manner. These solutions in 1:15 and 1:30 dilutions with distilled water were prepared from the clots and tested in ratios of 6:1, 12:1 and 18:1 (1:15 hemoglobin dilutions) and 12:1, 18:1 and 24:1 (1:30 hemoglobin dilutions) with antigen suspension prepared with 0.3 percent NaCl solution.

Taking the occurrence of precipitation in any one ratio as a positive reaction, then 30 per cent of hemoglobin solutions prepared from malarial clots and 5 percent from nonmalarial clots were found to be positive when aged in their own sera (Table 2). Hemoglobin solutions prepared from the same clots aged in saline showed 83 percent and 95 percent precipitation, respectively.

Table 2—Precipitation of Hemoglobin Solutions with Antigen Suspension when Hemoglobin Solutions are Prepared from Malarial and Nonmalarial Clots Aged in Serum or Saline

Hemoglobin solutions prepared from clots aged											
In serum						In saline					
Malarial			Nonmalarial			Malarial			Nonmalarial		
No. tested	No. Pos.	% Pos.	No. tested	No. Pos.	% Pos.	No. tested	No. Pos.	% Pos.	No. tested	No. Pos.	% Pos.
37	11	30	20	1	5	36	30	83	20	19	95

It is evident from these results that when portions of the same blood clots, malarial or nonmalarial, are aged in their own serum and the other portions aged in salt solution, the hemoglobin solutions prepared from those aged in salt solution have a much greater tendency to precipitate with tissue extract antigens than do those aged in serum. However, the hemoglobin solutions prepared under the latter conditions show an insignificant difference between malarial and nonmalarial clots, while hemoglobin solutions prepared from clots aged in serum show precipitation six times as frequently with malarial than with nonmalarial blood specimens.

2. *Effect of Using Different Concentrations of NaCl Solutions in the Preparation of Antigen Suspension and Hemoglobin Solution.*

It was observed that, if antigen suspension prepared with 0.85 percent NaCl solution instead of 0.3 percent is employed with hemoglobin solutions, the tendency toward precipitation is greatly reduced. For example, hemoglobin dilutions of 1:15 and 1:30 with water, mixed with antigen suspension prepared with 0.85 percent NaCl solution, show no precipitation, while when mixed with antigen suspension prepared with 0.3 percent NaCl solution generally show precipitation.

In order to determine the effect of several concentrations of salt solution used in the preparation of antigen suspension on the precipitation results, the following experiments were carried out.

A 1:15 hemoglobin dilution with water was prepared in the usual manner. A series of doubling dilutions were then prepared from the above dilution up to a ratio of 1:15360 with water. These dilutions were tested with antigen suspensions prepared with 0.3 percent and 0.85 percent salt solutions, respectively, in hemoglobin: antigen ratios of 1:4, 1:2, 1:1, 3:1, 6:1, 12:1 and 30:1. The results of this experiment are presented in Table 3.

It is evident that the zone of precipitation with 0.85 percent NaCl antigen suspension is markedly smaller than the zone obtained with 0.3 percent NaCl antigen suspension. It is of interest to note that precipitation did not occur in the hemoglobin:antigen suspension ratios of 1:4, 1:2 and 1:1 in any of the hemoglobin di

lutions when 0.85 percent salt solution was used in making the antigen suspension. In these ratios the final salt concentrations (not including the salt concentration in the hemoglobin solution) were approximately 0.7, 0.6 and 0.4 percent, respectively, which were shown later to be sufficient to inhibit precipitation. In the other hemoglobin dilutions in which no precipitation was noted, the hemoglobin:antigen ratios were the same in both, the 0.85 percent and 0.3 percent NaCl systems.

In the above experiment the effect of salt concentration in antigen suspension on hemoglobin precipitation was studied; in the following one, the amount of NaCl was kept constant in the antigen suspension and varied in the hemoglobin solution. Four hemoglobin dilutions were prepared in ratios of 1:15 with water. One of the four hemoglobin preparations was serially diluted up to 1:1920 with water and served as a control. Enough 20 percent NaCl solution was added to the other three hemoglobin preparations to make final concentrations of 0.1, 0.2, and 0.3 percent NaCl, respectively. These hemoglobin dilutions were then diluted serially up to 1.1920 with 0.1, 0.2, and 0.3 percent NaCl solution. In all cases a precipitate formed when 20 percent NaCl solution was

Table 3—Illustration of Zones of Precipitation Shown by Hemoglobin Solutions With Tissue Extract Antigen Prepared with 0.3% and 0.85% NaCl Solution

Dilution of hemoglobin with water	Hemoglobin dilution, cc								
	0.05	0.05	0.1	0.15	0.15	0.15	0.18	0.24	0.30
	Antigen suspension, cc								
	0.2	0.1	0.1	0.05	0.025	0.0125	0.01	0.01	0.01
	Ratios of hemoglobin dilution to antigen suspension								
	1:4	1:2	1:1	3:1	6:1	12:1	18:1	24:1	30:1
	Antigen suspension prepared with 0.3% NaCl solution								
1:15	4	4	--	-	-	-	-	-	-
1:30	4	4	4	-	-	--	-	-	-
1:60	4	4	4	4	-	-	-	-	-
1:120	4	4	4	4	4	4	4	4	-
1:240	-	4	4	4	4	4	4	4	4
1:480	-	4	4	4	4	4	4	4	4
1:960	-	-	4	4	4	4	4	4	4
1:1920	-	-	-	4	4	4	4	4	4
1:3840	-	-	-	-	4	4	4	4	4
1:7680	-	-	-	-	-	-	-	-	-
1:15360	-	-	-	-	-	-	-	-	-
	Antigen suspension prepared with 0.85% NaCl solution								
1:15	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-
1:120	-	-	-	-	-	-	-	-	-
1:240	-	-	-	4	4	-	-	-	-
1:480	-	-	-	4	4	4	4	4	4
1:960	-	-	-	4	4	4	4	4	4
1:1920	-	-	-	4	4	4	4	4	4
1:3840	-	-	-	-	4	4	4	4	4
1:7680	-	-	-	-	-	-	-	-	-
1:15360	-	-	-	-	-	-	-	-	-

added to the 1:15 hemoglobin dilution so that it was necessary to centrifuge each tube and remove the precipitates before the final dilutions were made. No spontaneous precipitation occurred in the dilutions. These dilutions were tested in ratios of 1:4, 1:2, 1:1, 3:1, 6:1, 12:1, 18:1, 24:1, and 30:1 with antigen suspension prepared with 0.3 percent sodium chloride solution.

A typical set of results is presented in Table 4. It is evident that the precipitation with hemoglobin dilutions prepared in 0.1 percent and 0.2 percent salt solutions occurred in narrower zones than in the controls, and that no precipitation occurred with hemoglobin dilutions prepared with 0.3 percent salt solution. It was

Table 4.—Effect of Employing Hemoglobin Dilutions Prepared in Various Concentrations of NaCl Solution on Precipitation with Antigen Suspension

Dilution of hemoglobin with water	Hemoglobin dilution, cc: 0.05	0.05	0.1	0.15	0.15	0.15	0.18	0.24	0.30
	Antigen suspension, cc: 0.2	0.1	0.1	0.05	0.025	0.0125	0.01	0.01	0.01
	Ratios of hemoglobin dilution to antigen suspension: 1:4	1:2	1:1	3:1	6:1	12:1	18:1	24:1	30:1
	Hemoglobin dilutions prepared with water								
1:15	--	--	--	4	1	--	--	--	--
1:30	4	2	--	4	2	--	--	--	--
1:60	4	4	4	4	4	4	--	--	--
1:120	4	4	4	4	4	4	--	--	--
1:240	4	4	4	4	4	4	4	4	4
1:480	4	4	4	4	4	4	4	4	4
1:960	4	4	4	4	4	4	4	4	4
1:1920	4	4	4	4	4	4	4	4	4
	Hemoglobin dilutions prepared with 0.1% NaCl solution								
1:15	--	--	--	4	--	--	--	--	--
1:30	4	--	--	4	4	--	--	--	--
1:60	4	4	--	4	4	--	--	--	--
1:120	4	4	--	4	4	--	--	--	--
1:240	4	4	4	4	4	4	--	--	--
1:480	4	4	4	4	4	4	4	--	--
1:960	4	4	4	4	4	4	4	4	4
1:1920	4	4	4	4	4	4	4	4	4
	Hemoglobin dilutions prepared with 0.2% NaCl solution								
1:15	--	--	--	--	--	--	--	--	--
1:30	--	--	--	--	--	--	--	--	--
1:60	2	--	--	4	--	--	--	--	--
1:120	4	--	--	4	--	--	--	--	--
1:240	4	4	--	4	4	--	--	--	--
1:480	4	4	4	4	4	--	--	--	--
1:960	4	4	4	2	2	--	--	--	--
1:1920	2	2	1	--	--	--	--	--	--
	Hemoglobin dilutions prepared with 0.3% NaCl solution								
1:15	--	--	--	--	--	--	--	--	--
1:30	--	--	--	--	--	--	--	--	--
1:60	--	--	--	--	--	--	--	--	--
1:120	--	--	--	--	--	--	--	--	--
1:240	--	--	--	--	--	--	--	--	--
1:480	--	--	--	--	--	--	--	--	--
1:960	--	--	--	--	--	--	--	--	--
1:1920	--	--	--	--	--	--	--	--	--

also observed that the precipitation reactions were similar with hemoglobin from malarial and nonmalarial blood specimens for each salt concentration.

The concentration of NaCl which will inhibit precipitation in mixtures of hemoglobin solution and antigen suspension is approximately 0.3 percent. This concentration exerts an inhibitory effect on precipitation regardless whether the salt has been added to the hemoglobin or to the antigen suspension. This inhibitory effect can be neutralized by the addition of sufficient water to the hemoglobin-antigen mixtures to reduce the salt concentration to less than 0.1 percent, except in those mixtures in which precipitation is weak even in the absence of added NaCl solution.

The effect of NaCl on the hemoglobin-antigen system is also illustrated by the following experiment. Hemoglobin dilutions were prepared by hemolyzing packed red blood cells in water, and in 0.1, 0.2 and 0.3 percent salt solution, respectively. These hemoglobin dilutions were then tested with antigen suspension. The results showed that the range of precipitation was reduced when the final salt concentration was about 0.12 percent and was completely inhibited when the final concentration was about 0.2 percent.

3. *Effect of Physical State of Antigen Suspension on the Formation of Precipitates with Hemoglobin Solution.*

It is well known that the physical state of the antigen suspension is of the utmost importance in tests for syphilis. For example, in the case of Kahn antigen, the antigen-salt solution mixture is of high sensitivity when in the form of a suspension of visible lipid aggregates. When in the form of an opalescent solution, sensitivity is greatly reduced. It seemed of interest to determine whether in the formation of precipitates with hemoglobin solution the same requirement as to the physical state of the antigen salt solution mixture holds true.

It was observed that opalescent solutions of antigens and salt solution give apparently the same degree of precipitation as suspensions of antigen and salt solution. A basic requirement is that the salt solution be of an NaCl concentration less than 0.85 percent. The following experiment illustrates this finding.

Into three series of 8 tubes each were measured 0.1 cc. of antigen suspension prepared with 0.1 percent NaCl solution. To each tube of the first series was added 0.1cc. of distilled water. To each tube of the second series was added 0.1 cc of 0.3 percent NaCl solution and to each tube of the third series was added 0.1cc. of

0.85 NaCl solution. The lipid aggregates in all of the 24 tubes had thus been dispersed and the antigen suspensions had the appearance of opalescent solutions. Serial dilutions of hemoglobin solution ranging from 1:15 to 1:1920 with distilled water were added in 0.1 cc. amounts to all the tubes and precipitation noted.

As is evident from Table 5, the tubes to which were added, respectively, 0.1 cc. amounts of distilled water and 0.3 percent NaCl solution, showed marked precipitation, except in the last tubes of the series which contained the 1:1920 hemoglobin dilutions. The tubes to which 0.1 cc. amounts of 0.85 percent NaCl solution was added showed no precipitation. Of interest is the fact that when 0.5 cc. amounts of water were added to these latter tubes, precipitation appeared in the tubes containing hemoglobin dilutions of 1:30 to 1:960, but not in the tubes containing hemoglobin dilutions of 1:15 and 1:1920.

The results indicate that, as already stated, the antigen-salt solution mixture can be in a state of an opalescent solution to give precipitation results with hemoglobin solutions. Also, that the addition of an equal amount of 0.85 percent NaCl solution to antigen suspension, prevents precipitation with hemoglobin solutions. Finally, if after thus preventing precipitation by the 0.85 percent NaCl solution, the salt concentration is reduced to about 0.15 percent by the addition of an appropriate amount of water, precipitation occurs.

Table 5. — Effect of Addition of Various Concentrations of NaCl Solution to Antigen Suspension on Precipitation with Hemoglobin Solution

Dilutions of hemoglobin with water							
1:15	1:30	1:60	1:120	1:240	1:480	1:960	1:1920
Amount of hemoglobin solution, cc							
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Amount of antigen suspension (0.3% NaCl solution)							
0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Precipitation results							
4	4	4	4	4	4	4	–
Addition of 0.1 cc amounts of water to each 0.1 cc of suspension before adding 0.1 cc hemoglobin solution							
Precipitation results							
4	4	4	4	4	4	4	–
Addition of 0.1 cc amounts of 0.3% NcCl solution to each 0.1 cc of antigen suspension before adding 0.1 cc hemoglobin solution							
Precipitation results							
4	4	4	4	4	4	4	–
Addition of 0.1 cc amounts of 0.85% NaCl solution to each 0.1 cc of antigen suspension before adding 0.1 cc of hemoglobin solution							
Precipitation results							
–	–	–	–	–	–	–	–
Addition of 0.5 cc amounts of water to each test above in which precipitation was prevented by the 0.85% NaCl solution							
Precipitation results							
–	4	4	4	4	4	4	–

4. *Effect of pH on Hemoglobin-Antigen Precipitation*

A study was made of the effect of pH on the precipitation reaction in mixtures of hemoglobin solution and antigen suspension. Most hemoglobin solutions prepared under routine conditions were found to be close to neutrality, the pH ranging from 6.9 to about 7.2. Acidification of the hemoglobin solution to pH 6.7 caused clouding of the solution and slight flocculation, apparently of a protein nature. At a pH 6.5 relatively heavy spontaneous flocculation was noted. Whenever such (protein) flocculation occurred, the floccules were removed by centrifugation before mixing the hemoglobin solution with antigen suspension. In the case of antigen suspension, the pH was generally about 5.5.

Portions of a hemoglobin solution of pH 7.2 giving negative reactions in 6:1 ratio with antigen suspensions were adjusted to pH levels ranging from 7.2 to 6.3 by addition of small amounts of 0.1 N HCl. The precipitation reaction with antigen suspension remained negative up to a pH of 6.7 and became strongly positive at a pH of 6.3.

A hemoglobin solution which originally showed a pH of 7.1 was then mixed with antigen suspension in 6 ratios; namely, 1:3, 1:1, 3:1, 6:1, 12:1, and 24:1. Table 6 presents the pH of the mixtures and the resulting precipitation reactions obtained. It is clear from the table that at a pH lower than 6.7, precipitation occurs, and at a pH higher than 6.7, no precipitation occurs. However, pH is evidently not the only factor responsible for precipitation of hemoglobin solution with antigen suspension.

Table 6. — Effect of pH on Precipitation in Mixtures of Hemoglobin and Antigen

Ratio of hemoglobin solution:antigen suspension					
1:3	1:1	3:1	6:1	12:1	24:1
pH of the mixtures of the hemoglobin:antigen					
5.9	6.4	6.7	6.8	7.0	7.1
Precipitation results					
4	4	4	–	–	–

5. *Solubility of Precipitates in Excess of Antigen or of Hemoglobin Solution.*

Precipitation reactions which occur on mixing hemoglobin solutions with antigen suspension apparently behave like antigen-antibody reactions and other collodial systems in respect to solubilty of precipitates in excess of antigen or antibody. If hemoglobin solution is mixed with antigen suspension in ratios outside of the optimal zone, no precipitation is obtained. It is possible that this lack of precipitation is due to an excess of antigen or of hemoglobin solution. The basis for this assumption is illustrated in Table 7.

Table 7. — Solubility of Precipitates in Excess of Antigen Suspension or of Hemoglobin Solution

Ratios of hemoglobin solution:antigen suspension								
1:4	1:2	1:1	3:1	6:1	12:1	18:1	24:1	30:1
Effect of an excess of hemoglobin solution								
Precipitation results								
4	4	4	4	4	–	–	–	–
Precipitation results after changing second, third, fourth and fifth ratios to 12:1 by addition of hemoglobin solution								
4	–	–	–	–				
Effect of an excess of antigen suspension								
Precipitation results								
–	–	–	3	4	4	4	4	4
Precipitation results after changing fourth to eight ratios to 1:1 by addition to antigen suspension								
			–	–	–	–	–	4

Nine ratios of hemoglobin solution:antigen suspension were set up as follows: 1:4, 1:2, 1:1, 3:1, 6:1, 12:1, 18:1, 24:1, and 30:1. The amounts of hemoglobin and antigen suspension were the same as those given in Table 2. Precipitation was noted in ratios 1:4, 1:2, 1:1, 3:1 and 6:1 and not in ratios 12:1 to 30:1. The following amounts of hemoglobin solutions, respectively, were then added to the tubes containing the 1:2, 1:1, 3:1 and 6:1 ratios: 1.15 cc., 1.1 cc., 0.45cc., and 0.15 cc. These amounts brought the hemoglobin-antigen ratio in these tubes to 12:1 and the precipitates in these tubes immediately dispersed.

A similar experiment with another hemoglobin solution was carried out. Nine ratios of the hemoglobin solution-antigen suspension ranging from 1:4 to 30:1 was set up. Precipitation was noted in the tubes containing the following ratios: 3:1, 6:1, 12:1, 18:1, 24:1, and 30:1. The following amounts of antigen suspension were then added to the above tubes, 0.1 cc., 0.125 cc., 0.14 cc., 0.17 cc, 0.23 cc. and 0.29. cc., bringing the hemoglobin:antigen ratio to 1:1. The precipitates immediately dispersed.

These experiments indicate that precipitates in mixtures of hemoglobin solution and antigen suspension can be readily dissolved if an appropriate amount of hemoglobin solution or antigen suspension is added in excess of optimal requirements for precipitation. Further, in ratios that are not optimal, the addition of either antigen suspension or hemoglobin solution sufficient to render the ratio optimal will call forth precipitation. The antigen suspension employed in these experiments was prepared by mixing antigen with 0.3 NaCl solution.

6. *Effect of Shaking Hemoglobin Solution*

Shaking of the hemoglobin solutions (1:15 with distilled water) for 20 minutes in a Kahn shaking machine resulted in the forma-

tion of a moderately heavy white precipitate which, when suspended in 1.0 or 2.0 cc. of distilled water was found to be partially soluble. The soluble portion gave positive precipitation results with antigen suspension without respect to whether the unshaken portion of the hemoglobin solution had given positive or negative results with the same antigen suspension. This soluble portion gave positive precipitation results with antigen suspensions prepared with 0.3 as well as with 0.85 percent NaCl solution. The precipitate obtained on shaking of the hemoglobin solution was found to be readily soluble in 0.01 N-HCl solution. This finding combined with the fact that antigen suspensions prepared with 0.85 percent NaCl solution call forth precipitation of this shaken fraction of hemoglobin solution would suggest that this fraction might consist of globin. Globin, as is well known, is soluble in weak acid. It will also be seen below that antigen suspension prepared with 0.85 percent solution gives precipitation results with globin solutions. It should be added that no differences were noted between the shaken fractions of hemoglobin from malarial and nonmalarial blood speciment.

7. *Precipitation Reactions with Hemin, Globin, Ferric Chloride and potassium Chloride with Antigen Suspension.*

An effort was made to determine which substance in hemolyzed red blood cells is responsible for the precipitation observed when hemoglobin solution is mixed with antigen suspension. The substances examined for their reactions were hemin, globin, iron in the form of ferric chloride and potassium chloride. The antigen suspensions in these studies were prepared with 0.3 percent NaCl solutions.

Hemin and Globin. Hemoglobin from cattle, giving positive precipitation results with antigen suspension, was split into globin and chromogen in order to find which of these two substances is responsible for percipitation with antigen suspension. The method of splitting was that of Anson and Mirsky.[2] It was found that the chromogen in serial doubling dilutions with distilled water ranging from 1:5 to 1:80 gave negative precipitation reactions with antigen in ratios of 1:1, 3:1, 6:1 and 12:1. The globin fractions in the same dilutions with distilled water and in the same ratios with antigen suspension gave positive precipitation results.

Commercial preparations of hemin (recrystallized) and globin* were then tested with antigen suspension. The hemin was diluted

* Obtained through the courtesy of Eli Lilley Research Laboratories and Wellcome Research Laboratories.

[2] Anson, M. L., and Minsky, A. E. 1929. Protein Coagulation and its Reversal. The Preparation of Insoluble Globin and Heme. J. Gen. Physiol. 13, 469.

1:4 through 1:256 with distilled water and tested with antigen suspension in ratios of 1:1, 3:1, 6:1 and 12:1. No precipitation occurred. The globin in the form of globin hydrochloride in a 1 percent solution in water, was found to give positive precipitation results in a 6:1 ratio with antigen suspension up to a dilution of 1:128 with water. The pH of the globin was 2.7.

A 1 percent globin hydrochloride solution in water was neutralized with 0.1N NaOH and brought to pH 7.1. Beginning at pH 6.5 a heavy (protein) precipitate appeared which was removed by centrifugation. The supernatant was diluted 1:4 through 1:256 with water and mixed with antigen suspension in a 6:1 ratio and was found to give precipitation reactions up to a 1:64 dilution.

The precipitation reaction of globin solution with antigen suspension is apparently not inhibited when the globin solution is prepared with 0.85 percent NaCl solution instead of with water. As was seen above, in the case of hemoglobin solution, the use of 0.85 percent NaCl solution in the preparation of antigen suspension inhibits precipitation.

Ferric chloride. When a 10 percent ferric chloride solution is diluted from 1:15 through 1:3840 with water and then mixed with 0.3 percent antigen suspension in ratios from 1:4 to 30:1, the precipitation reaction pattern closely resembles that which is obtained upon using hemoglobin solution. When a 1 percent solution of ferric chloride is used as the base for preparing dilutions for a similar test, the occurrence of precipitation is limited to a very narrow zone. corresponds to that found in one of the above dilutions of the one percent base solution, one might conclude that iron, *per se*, is not the agent responsible for the precipitation observed in mixtures of hemoglobin solution and antigen suspension.

Another experiment, with 10 percent ferric chloride diluted 1:15, 1:30, and 1:60 in 0.85 percent NaCl solution and tested with 0.3 percent antigen suspension in ratios of 3:1 through 30:1 showed precipitation in each tube. Since negative results are obtained if hemoglobin solution is diluted with 0.85 percent NaCl solution, this would add support to the above observation that iron, as present in ferric chloride, is not the responsible precipitating agent.

The effect of potassium, as present in KCl solution, on precipitation of antigen suspension was also studied. The first experiments were performed in the same way as with ferric chloride. A 0.75 percent solution of KCl was prepared and from this, dilutions were made in water ranging from 1:15 through 1:7680. This range was employed in order to include the concentration of potassium in

hemoglobin solutions. Each dilution was tested with 0.3 percent NaCl antigen suspension in ratios from 1:4 through 30:1. None of these tubes showed precipitation.

An attempt was then made to determine the minimum concentration of potassium chloride that will precipitate with 0.3 percent NaCl antigen suspension. A 10 percent solution of KCl was prepared and serially diluted with water to make dilutions of 5, 2.5, 1.25, 0.625, and 0.312 percent. These dilutions were tested with antigen suspension in ratios of 3:1, 6:1, and 12:1. Positive results were obtained with 10, 5, and 2.5 percent of the KCl dilutions but not with the higher dilutions. When the KCl dilutions were tested with an antigen suspension prepared with 0.85 percent NaCl solution (instead of 0.3 percent) precipitation occurs with the 5 percent dilution but not with the 2.5 percent. These results indicate that potassium, *per se,* is not the responsibile precipitating agent in the hemoglobin solution.

Summary

Studies were carried out on the precipitation reaction occurring when hemoglobin solution (laked erythrocytes) is mixed with lipid (Kahn) antigen.

1. It was observed that, when hemoglobin solutions were prepared from blood clots aged in saline and in serum, there were marked differences in the precipitation results. Hemoglobin solutions prepared from clots aged in saline showed 80 to 95 percent reactivity with lipid antigen suspension and no differentiation between hemoglobin of malarial and nonmalarial origin. Hemoglobin solutions prepared from clots aged in serum showed 30 percent reactivity with hemoglobin of malarial origin and 5 percent reactivity with hemoglobin of nonmalarial origin.

2. Hemoglobin solutions when mixed with lipid antigen suspension prepared with 0.85 percent NaCl showed considerably less precipitation than when mixed with antigen suspension prepared with 0.3 percent NaCl solution. Hemoglobin dilutions with water extending from 1:15 to 1:120 showed precipitation with antigen suspension prepared with 0.3 percent NaCl solution but not with 0.85 percent.

3. When the hemoglobin solution was prepared with 0.1 or 0.2 percent NaCl solutions, precipitation with lipid antigen suspension occurred in narrower zones than in controls in which the hemoglobin solution was prepared with water. When the hemoglobin solution was prepared with 0.3 percent NaCl solution, no precipitation occurred with antigen suspension.

4. The mixture of lipid antigen and 0.3 percent salt solution need not be in the form of a suspension of lipid particles to call forth precipitation with hemoglobin solution; the mixture in the form of an opalescent solution calls forth similar precipitation.

5. The addition of an equal amount of 0.85 percent NaCl solution to antigen suspension prepared with 0.3 percent salt solution will prevent precipitation with hemoglobin solution, but the addition of an appropriate amount of water to the same hemoglobin-antigen mixture, enough to reduce the salt concentration to about 0.15 percent, will elicit precipitation.

6. The hemoglobin precipitation reaction is apparently affected by acid similarly to other precipitation and agglutination reactions. Most hemoglobin solutions prepared under routine conditions were found to be close to neutrality, the pH ranging from 6.9 to 7.2. When hemoglobin solution which gave no precipitation reaction with antigen suspension in a 6:1 ratio was acidified with 0.1 N HCl and was mixed with the suspension, precipitation was observed in the same ratio when the pH was 6.7 and lower and not when the pH was 6.8 and above.

7. Precipitation reactions of hemoglobin solution and antigen suspension apparently behave like antigen-antibody reactions in respect to solubility of the precipitates in excess of antigen or antibody. The precipitates in the hemogobin reactions are readily soluble in a relative excess of hemoglobin solution or of antigen suspension.

8. Shaking of the hemoglobin solution causes the separation of a white floccule which appears to be globin. This floccule is partially soluble in distilled water, and when this solution is mixed with antigen suspension prepared either with 0.3 or with 0.85 percent salt solution, a precipitate results.

9. Commercial preparation of hemin and globin, in the form of globin hydrochloride, were tested with lipid antigen suspension. Precipitation was noted with the globin but not with the hemin.

INADEQUATE HOUSE SCREENING AS A FACTOR IN MALARIA TRANSMISSION

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(Received for Publication 26 November 1945)

The screening of houses and the use of bed-nets, as measures to prevent malaria and other insect-borne diseases, have long been practiced. Success in the use of these methods depends on intelligent and painstaking care to make them effective. Improper use of either can convert them into mosquito traps, as is especially evident when they are first introduced to populations not experienced in their use.

An intelligent use of house screening demands that all possible places where mosquitoes can enter the house should be screened or sealed, that doors be so made that mosquitoes do not enter, that doors be kept closed, particularly during flight hours, and that mosquitoes which have gained entrance be killed by "flitting" or "swatting." To learn such technique requires interest, conviction that the method is effective, and a certain amount of health education.

In the Tropics we often see screened barracks provided for labourers who have received no health instruction. Even when the original screening has been correctly done, one finds a tendency to keep doors open and to neglect the repair of damaged screens, and but little interest in, or provision for, killing mosquitoes which have gained entrance.

In the course of a medical survey in May 1943 at an industrial (mining) settlement on the Berbice River, about 140 miles from the Atlantic coast in British Guiana, the writer observed some exceptionally high malaria infection rates in mosquitoes trapped in houses where such conditions existed. The workers lived in screened wooden houses and in temporary open camps. Inspection of the screened houses revealed numerous cracks and other openings in the floors, walls, and roofs, and it was found that the doors were often left open after sunset. Hundreds of mosquitoes were captured in the houses; 83 per cent of them were anophelines and 17 per cent were culicines. Only two species of the former were present, 85 per cent being *Anopheles darlingi* Root and 15 per cent *A. "tarsimaculatus."*[1]

Dissection of the mid-guts of female anophelines captured in the inadequately screened houses revealed surprisingly high infec-

[1] See p. 225 *et seq.*

tion rates. Oöcysts were found in 33 per cent of *A. "tarsimaculatus"* and in 88.8 per cent of *A. darlingi*. Blood examinations of the tenants of the houses showed that 36 per cent had malaria infections, with a very high rate of gametocyte carriers.

After the institution of routine daily mosquito captures in the houses and the treatment of human carriers, the percentage of infected mosquitoes dropped to less than 1 per cent in a very short time, even though the buildings could not be repaired immediately and considerable time was required to enforce the closing of doors before sunset.

Captures and Dissections Made Before Institutions of Control Measures Mosquito captures

Mosquito captures

		Number
Total Anophelines		3486
A. darlingi	2963	
A. "tarsimaculatus	523	
Total Culicine Species		714

Mosquito dissections

	No. dissected	No. with oöcysts	Per cent positive
A. darlingi	250	222	88.8
A. "tarsimaculatus"	57	19	33.3

Dissections After Institution of Control Measures—Two Months Later

	No. dissected	No. with oöcysts	Per cent positive
A. darlingi	305	3	0.98
A. "tarsimaculatus"	10	0	0

The high infection rates found among the anophelines captured in the screened houses must be considered abnormally great, and not such as would be encountered under natural conditions. Screened houses, in which large numbers of gametocyte carriers were present, played the role of mosquito traps. The conditions under which the high rates existed were therefore those of an experimental infection carried out under ideal circumstances, since the conditions of temperature and humidity were those of the natural environment, and no artificial handling was necessary.

The very high rate of infection in the anopheline mid-guts, and the diversity in the sizes of the oöcysts, suggest that the mosquitoes were trapped for a long time and frequently reinfected. Huff (1930) has shown that susceptible mosquitoes may be infected more than once, and Boyd, Kitchen and Kupper (1937) have demonstrated that an anopheline can be simultaneously infected by two different species of malaria parasites.

Equipment and facilities available at the time of the observations here reported were insufficient to ascertain sporozoite infection rates, but *A. darlingi* is well known to be an extremely efficient transmitter of malaria. The rapid decrease in malaria incidence after routine mosquito captures were started confirms our belief that there was a good agreement between the percentages of stomach and salivary gland infections.

Discussion

The percentage of anopheles infected in nature varies with the species and its habits, such as domesticity, and is dependent on special conditions such as flight range, availability of infected blood, the susceptibility of the mosquito to infection by malaria parasites, and the ability of the parasites to complete the cycle of development in the mosquito. Experimental infections of anophelines under suitable conditions may produce high infection rates: for example, Mayne and Griffitts (1931) infected 85.7 per cent of *A. atropos* under such conditions. Natural infections vary considerably, but seldom reach the high rates obtained under experimental conditions.

A darlingi Root, is known to be an efficient malaria vector. Bennaroch (1931) found 10.5 per cent of this species naturally infected in Venezuela, and in Brazil Shannon (1933) found 9 per cent, Davis (1931) 22 per cent, Davis and Kumm (1932) 28.7 per cent, and Kumm (1932) 60 per cent in different localities. On the coast of British Guiana and in the interior of Surinam (1942) it is the principal vector of malaria. As previously observed by Giglioli (1938), *A. darlingi* is a domestic species which tends to remain in the neighbourhood of its feeding place. Under the conditions mentioned above, even *A. "tarsimaculatus"* behaved in the same way.

The name "tarsimaculatus" has been used here, as there is still doubt among some taxonomists as to whether the local species is *A. aquasalis* Curry, although it appears to be indistinguishable morphologically. The species present may be *A. oswaldoi*, which closely resembles *A. aquasalis*. The important role of aquasalis as a malaria vector was established by De Verteuil (1933) in Trinidad, by Earle (1936) in Grenada and St. Lucia, and by Tournier (1937) in French Guiana. On the contrary Giglioli (1938), who intensively studied local malaria vectors, denies to this species any importance in the transmission of human malaria in British Guiana, where the principal, if not the only vector, is *A. darlingi*. The observations suggest that it is possible under artificial conditions to cause a species of anophelines to become an effective vector, and it is to be feared that such adaptation may become permanent in a species displaying

the adaptability demonstrated by our "tarsimaculatus." Some observers regard this species as being primarily zoophilic and are of the opinion that it attacks man only where insufficient animals are present to deviate it to them.

Summary

House screening, intended to prevent the entrance of anopheline mosquitoes, if inadequate or improperly used, may increase rather than decrease the malaria hazard. Mosquitoes, if permitted to gain entrance to houses, may remain virtually trapped, and by repeated biting of gametocyte carriers in these habitations, may build up extraordinarily high infection rates.

In a mining community in the interior of British Guiana, studies were made in barracks with inadequate or damaged screening, inhabited by a population untutored in the use of screening. In mosquitoes captured in the dwellings, oöcysts were found on the stomachs of 88.8 per cent of *Anopheles darlingi,* which is recognized to be an extremely efficient malaria vector, and in 33 per cent of *A. "tarsimaculatus" (aquasalis?),* which locally is considered not to be an important transmitter of malaria.

After the institution of the routine capture of mosquitoes which entered the barracks, and treatment of human malaria carriers, the oöcyst rate in *A. darlingi* fell to 0.98 per cent of 305 specimens dissected, and to zero in 10 specimens of *A. "tarsimaculatus."*

These findings emphasize the need for adequate house screening, for the proper use of screened houses, and for prompt destruction of mosquitoes in houses to which they have gained access.

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EXTENT OF MALARIA RESEARCH (Continued)

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New York

The vaccine studies carried out by Dr. Michael Heidelberger, using *Plasmodium vivax,* failed to reveal any protective action against either blood-induced or sporozoite-induced infections.

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(Col. H. W. Mulligan)

A special program, called "The Mammalian Malaria Enquiry," representing collaboration of the Royal Society of London, the Government of India, and the Indian Research Fund Association, has been under way since May 15, 1945.

The purpose of the program is to determine whether or not an exo-erythrocytic cycle can be demonstrated in mammalian malaria. Work is carried on with *P. cynomolgi* in rhesus monkeys. Sporozoite infections are available in *Anopheles annularis* and *A. subpictus.*

It is expected that the study will continue until March 31, 1947.

REVISED TRANSLATION—MEXICO HONORS DR. MARK F. BOYD

On page 139 of the June, 1946 issue of the Journal, there appeared a translation from the December, 1945 issue of Revista del Instituto *de Colubridad y Enfermedades Tropicales* of Mexico which should have read as follows:

> "The Institute of Public Health and Tropical Diseases, as well as various prominent malariologists, unite in tribute to Dr. Mark F. Boyd, for his valuable original contributions, teachings, and his sound judgment relating to the control of malaria."

REVIEW OF RECENT DEVELOPMENTS ON THE EPIDEMIOLOGY OF MALARIA*

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(Received for publication 14 November 1945)

Chief interest in the epidemiology of malaria in this country during the past year has been its incidence in the military personnel and the effects of the returning soldier or veteran with malaria contracted overseas upon the prevalence of the disease in this country. Malaria in the civilian population in this country has reached a record low prevalence. Likewise, the prevalence of malaria in the military personnel contracted in continental United States has been extremely low.

The invasion of more malaria-infested Pacific Islands by larger numbers of troops has allowed a large number of overseas admissions for malaria. Within the past year State Health Officer's reports indicate that admission to hospitals of military personnel with malaria contracted outside continental United States has equalled or exceeded that in the civilian population in almost all States. With the close of the War, those many thousands of veterans with malaria contracted overseas will be discharged to return to their communities in all the States.

The vast majority of this malaria is *vivax* malaria from the Pacific Area. It is reported to differ from the American *vivax* in its tendency to frequent relapses, its poor response to therapy, and its high rate of infection in the American negro. In one experience it was found to be most infective between the sixth and fifteenth relapse or eighty to twenty-one months after the initial attack, that the individual with asymptomatic parasitemia was more likely to infect mosquitoes than those clinically ill, and that treatment did not affect the rate of infection of mosquitos. There will be then for the next two or three years veterans returning to their communities who have foreign contracted malaria and who are infective to mosquitoes. The American Anopheline transmitters of malaria, *Anopheles quadrimaculatus* Say, and *Anopheles maculipennis freeborni* have been infected with foreign *vivax* strains of malaria and shown to be capable

* Report of the Committee on Epidemiology presented at the annual meeting of the National Malaria Society, Cincinnati, Ohio, 14 November 1945.

of transmitting it to susceptible persons. Seven and four-tenths per cent of mosquitoes in one study fed on cases of malaria with no gametocytes demonstrable microscopically in the peripheal blood became infected.

Under such circumstances it is possible for epidemic or endemic foci to be established by the returning veteran with malaria but modern methods of control should prevent any widespread distribution of the disease. For more than a year, there have been large numbers of soldiers with malaria home on furlough and being discharged in increasing numbers and few reports of increase in epidemic or endemic malaria has occurred in the communities to which they returned. It has been reported that *P. vivax* in the South Pacific was equally infective for the American negro with the white and that the returning negro veteran might create a problem in negro populations by introducing a new strain to which the adult negro is susceptible. County-wide thick film blood smear surveys in 23 counties in South Carolina, 1937-1943, showed that negro school children in that State had *P. vivax* parasitemia in a ratio of three negro to four white positive children for that species. This seems to indicate that in this country the young negro may not be as resistant to *vivax* infection as the adult negro. The studies also showed that for both races in South Carolina *P. falciparum* and *P. malarie* infections were more frequent that *P. vivax*. Area has been shown to be highly significant in malaria infections and would doubtless play an important role in any new foci that might develop.

Malaria in some of the invaded countries increased during the war. It was reported that 87 per cent of the population of Greece was infected and that 100 per cent of the people of some of the Pontine marsh provinces of Italy were infected. The island of Guam in the Pacific is reported to be entirely free of malaria. Reports from other European and Asiatic countries during the past year have been meager or absent.

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